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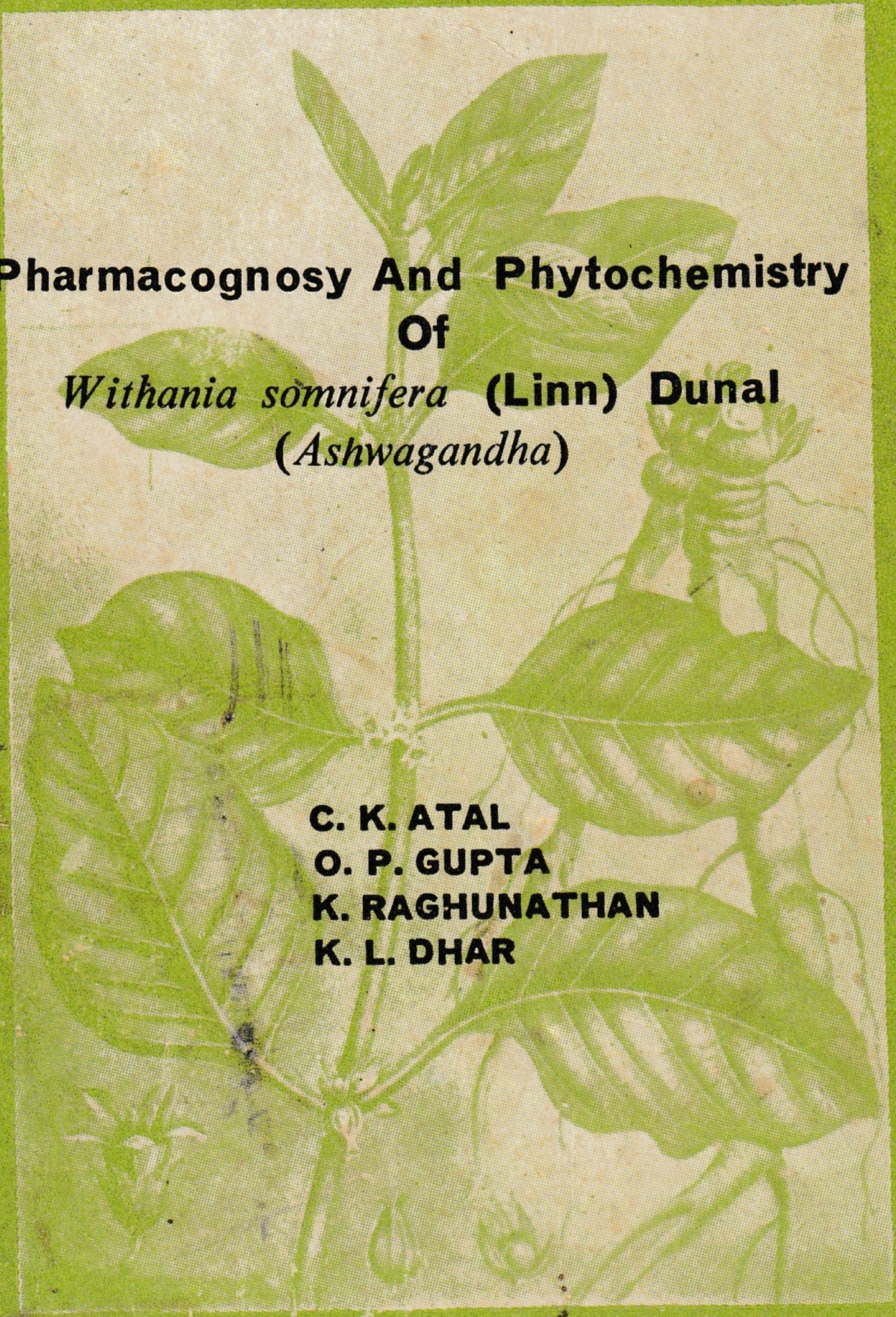

Pharmacognosy And Phytochemistry Of *Withania somnifera* (Linn) Dunal (*Ashwagandha*)

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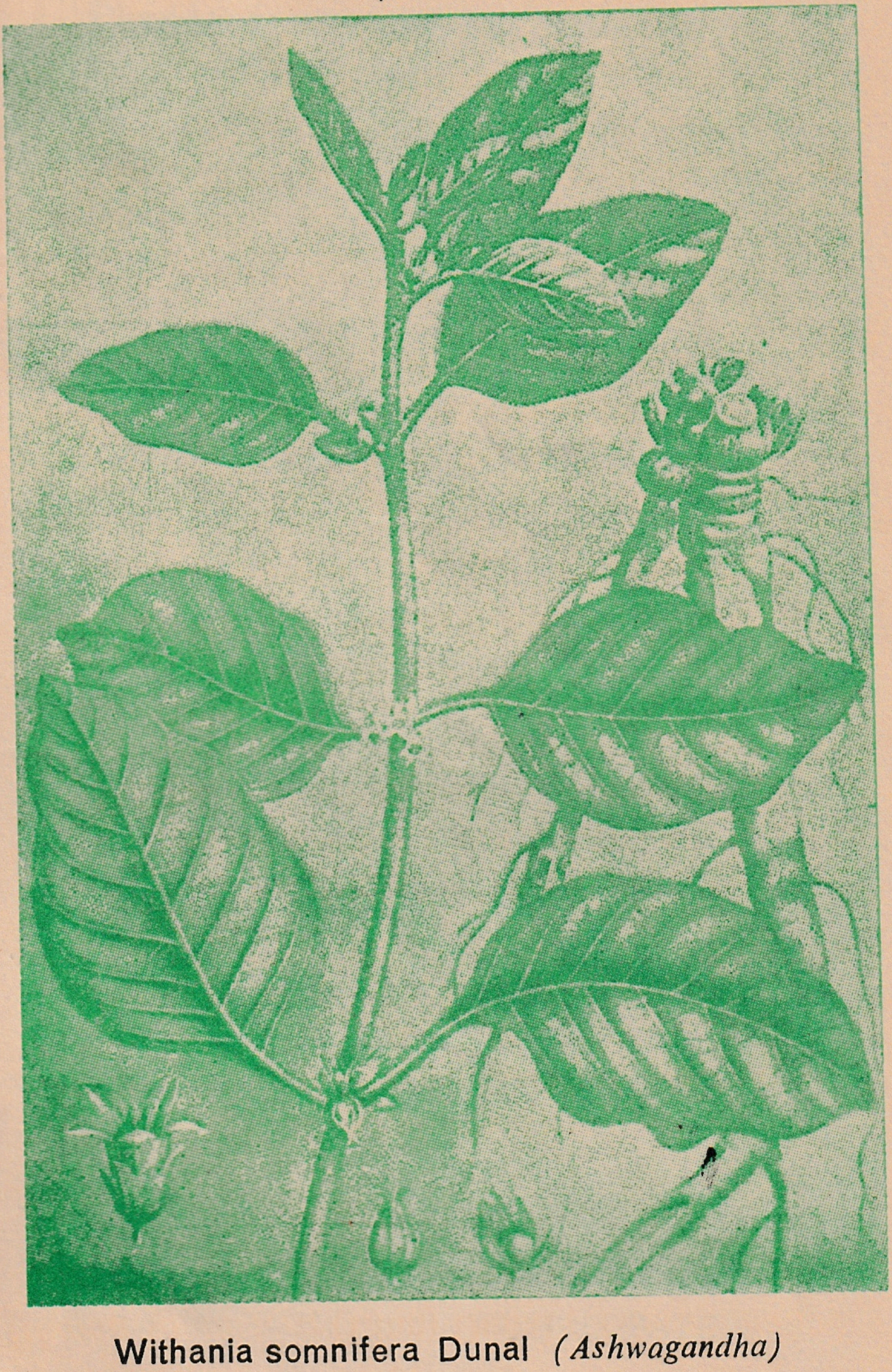

Withania somnifera Dunal *(Ashwagandha)*

Pharmacognosy and Phytochemistry of *Withania somnifera* (Linn) Dunal *(Ashwagandha)*

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1975

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Prologue

The monograph 'Pharmacognosy and phytochemistry of *Ashwagandha* (*Withania somnifera* (Linn.) Dunal), prepared by scientists of the Council provides a detailed picture on the work done on the drug '*Ashwagandha.*' It is an important drug in Indian Medicine used widely by physicians as single drug as well as in combination with other drugs since time immemorial.

The present monograph presents useful and valuable pharmacognostic and phytochemical data and I have all hope that scientists, research workers and academicians connected with this kind of work will consider this as a welcome addition to their libraries. The taxonomic information provided will help in establishing identity of the drug available in different markets and the phytochemical information is expected to open new vistas for understanding the structure-function relationship so that the areas of utility of the drug may be enlarged.

P.N.V. KURUP
DIRECTOR

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GENERAL : GEOGRAPHICAL AND OTHER ASPECTS

Pharmacognosy and Phytochemistry of *Ashwagandha* (*Withania somnifera* (Linn.) Dunal)

Withania somnifera (Linn.) Dunal commonly known as "*Ashwagandha*" belongs to the family Solanaceae. Family Solanaceae has got 1250 species widely distributed in the warmer parts of whole world. The genus *Withania* is reported to have 23 species and out of which i.e. *Withania somnifera* (Linn.) Dunal and *Withania coagulans* Dunal are of high medicinal value.

The former is a household remedy and therefore is known by various local names[8]. *Sanskrit*: Ashvagandha; *Hindi*: Asgund, Panir; *Tamil*: Amukkasi, Ashuhan; *Telugu*: Ashwagandha, Dommadolu; *Urdu*: Asgandhu, Gaori; *Bengali*: Ashwagandha; *Punjabi*: Ak, Aksan, Asgund, Asgund Nagori, Isgand; *Bombay;* Asgund, Asvagandha; *Arab*: Kakanaje hindi, *Persian:* Kaknajihindi,

Withania somnifera (Linn.) Dunal (*Ashwagandha*) is a reputed medicinal plant and is very much used in Indian System of medicine. It has been in use since the time of Punarvasu Atreya.

The various preparations and forms of "*Ashwagandha*" i.e. powder, decoction, oil, smoke, poultice etc. have been suggested for the cure of various diseases, such as leprosy, nervous disorders, intestinal affections, venereal diseases, rheumatism. emaciation of children and as a tonic for all kinds of weakness and also to promote vigour and stamina.

Geographical distribution:

Withania somnifera (Linn.) Dunal is cosmopolition and grows throughout the drier parts and sub-tropical India. It is widely distributed in North-Western India, Bombay, Gujarat, Rajasthan, Madhya Pradesh, Uttar Pradesh, Punjab plains extending to the mountain regions of Punjab, Himachal Pradesh and Jammu, ascending to a height of 1500 m.

The wild growth of this species has also been reported from Pakistan, Afganistan, Palestine, Egypt, Jordan, Morocco, Spain, Canary Island, Eastern Africa Congo, Madagascar and South Africa. These areas represent wide variations of soil, rainfall, temperature and altitude.

Description of the plant:

The morphological variations reported by Atal *et al*[6] in their detailed study of various forms collected from different regions i.e. Manasa, Pilani, Chandigarh, Delhi and Ahmedabad, are described herewith.

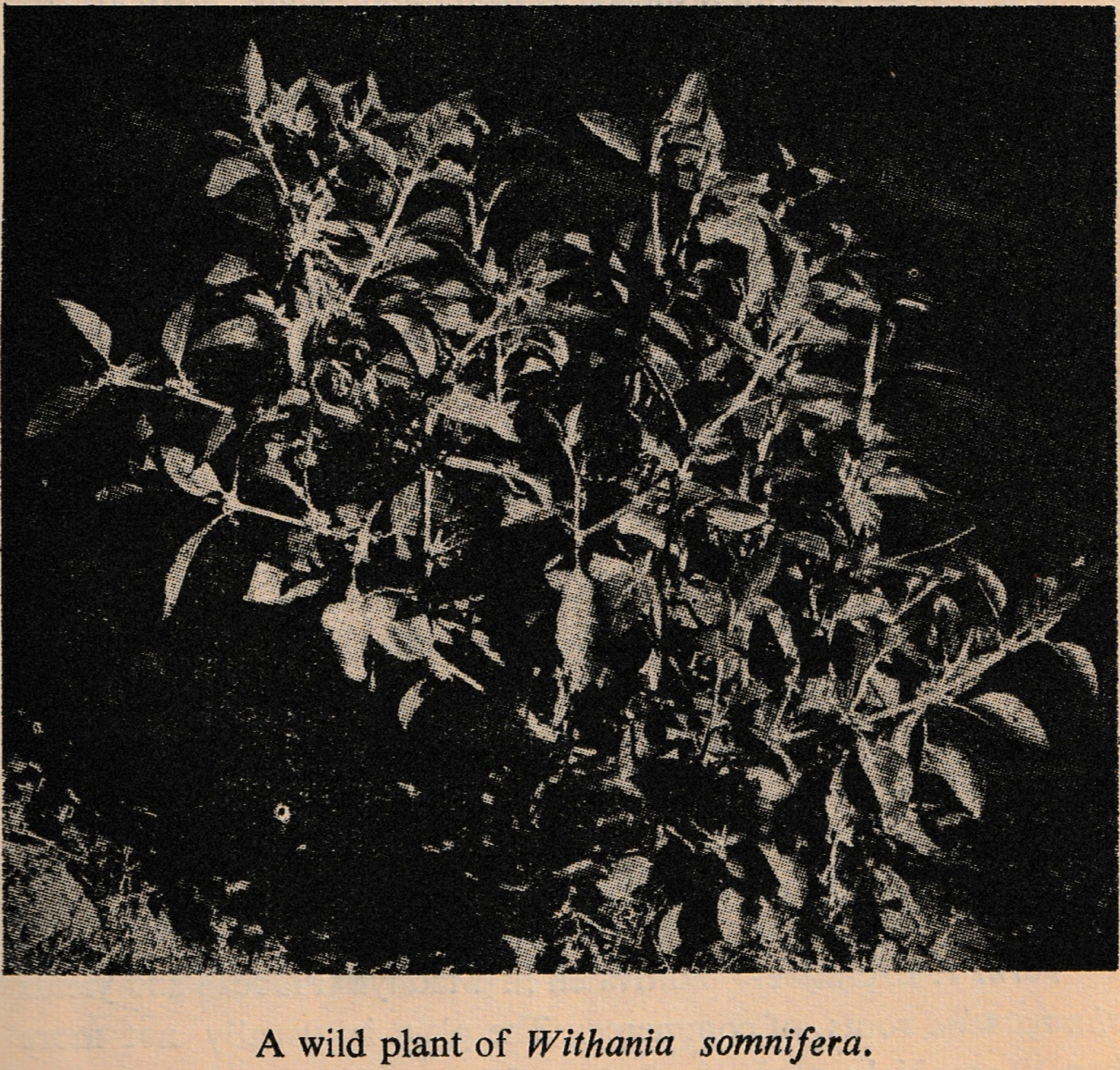
General description of a wild plant:[5]

The plant is usually erect, branched, unarmed shrub, upto 1.25 meters in height (Figure 1). The parts above the ground, specially the stem, the veins, and the calyx, are covered with a sparsely hairy tomentum. Branches round; leaves simple, petiolate, ovate, entire, exstipulate, acute, glabrous and upto 10 cm. long; petioles upto 1.25 cm. long. The leaves on vegetative shoots are alternate and large while those on floral branches are opposite, arranged in pairs of one large and one small leaf and arranged somewhat laterally, having in their axil a cymose cluster of 5-25 inconspicuous pale green flowers. Flowers short-pedicellate, 4-6 mm. in diameter, gamosepalous. sepals 5-parted. persistent, lobes linear acute. In the fruiting stage the calyx becomes enlarged, inflated, completely enclosing the fruite. Corolla 5-lobed, gamo-petalous, lobes spreading or recurved, acute, pubescent and greenish-yellow; Stamens 5, attached near the base of the corolla, epipetalous; anthers oblong, dehiscing longtiudinally; filaments linear-slender. Gynoecium syncarpous composed of minute swollen ovary subtended by a long slender style; stigmas shortly 2-fid. Fruite, a berry enclosed in the green presistent calyx, 5 mm. in diameter, green when unripe, orange-red when mature, containing numerous small capsicum-like seeds.

The cultivated plants are quite distinct in morphological characters from wild plants. According to Kaul[17] "the cultivated plants are different from the wild ones not only in their therapeutic properties but in all morphological characters like roots, stems, leaves, flowers, pollen grains, mature fruits, seeds and the enlarged calyx". He has also suggested the name *Withania ashwagandha* for cultivated plants. *Atal's*[5] observations agree with those of Kaul but he failed to find an enlarged calyx in the cultivated plants.

He has observed that the calyx in cultivated plants is not even one half of the enlarged or inflated calyx found in wild plants growing at the Punjab University Campus.

Figure 1



A wild plant of *Withania somnifera.*

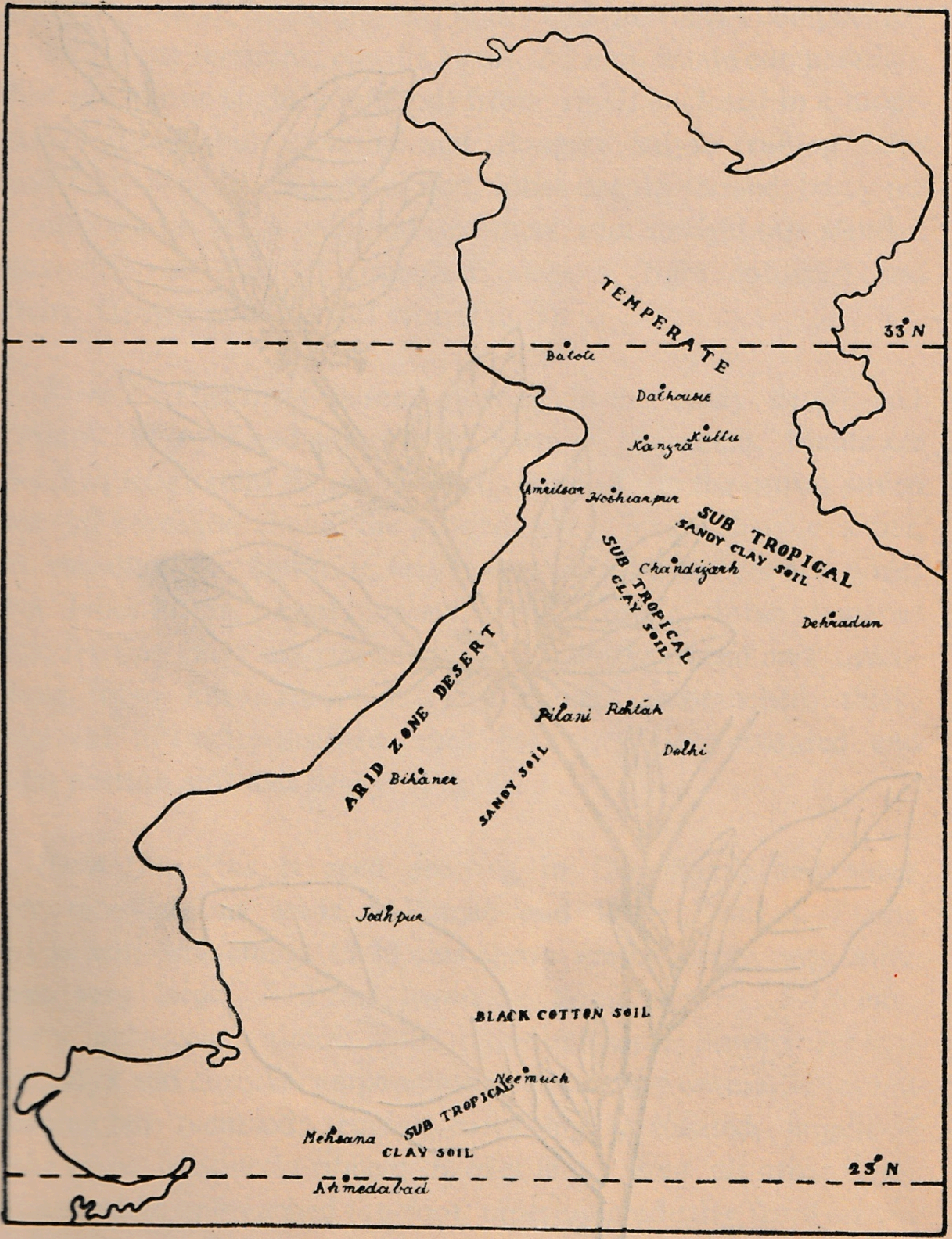
Intraspecfic variability in *Withania somnifera*:

Hooker[16] reported its distribution throughout drier subtropical areas of India, while Stewart[17] made only a casual mention of a red and white variety of *W. somnifera* growing in Punjab. Kaul[17] found differences in morphology between the cultivated and wild varieties and named the former as *W. ashwagandha* and the latter as *W. somnifera*. Out of twenty three species[3] of *Withania* reported in the world, only *W. simonii* is regarded synonymous of *W. somnifera*. It is intersting to note that the species of *Withania* have been differentiated on the basis of apparently minor characters. For example *W. obtusifolia* V. Tackholm is distinguished by leaves which are membraneous and show an obtuse apex in contrast to a somewhat thicker texture and acute apex of the leaves of *W. somnifera*. The name *W. ashwagandha* suggested for cultivated variety has no justification as a separate species, although it has been used in scientific writing,[48],[50],[10] but can at the most be considered as a variety *ashwagandha* of *W. somnifera*.

As mentioned earlier *W. somnifera* has a very wide geographical range. 1n India its range extends from 23° N to 33° N and extending from 180 m to 1500 m alt. This region is extremely diversified geologicaly, phytogeographically, climatically and edaphically. In order to know the difference amongst the plants growing in different localities, an extensive study of *W. somnifera* Dunal was made in the field in India (Fig. 2) at the Herbarium of Forest Research Institute, Dehradun and the Gray Herbarium at Harvard University by Atal and Schwarting[6]. A great variation with respect to the growth habit and morphological characters of plants population from India as well as from other countries was observed. The details of morphological characters of five different froms *W. somnifera* are described below:—

Form 1: Exclusively cultivated in Madhya Pradesh and yields *ashwagandha* roots of commerce. The plant is usually not more than 30 cm. high; number of stems from crown vary from one to

Figure 2


TEMPERATE
33°N
Balole
Dalhousie
Kangra
Kullu
Amritsar
Hoshiarpur
SUB TROPICAL
SANDY CLAY SOIL
Chandigarh
SUB TROPICAL
CLAY SOIL
Dehradun
ARID ZONE DESERT
Pilani
Rohtak
Delhi
Bikaner
SANDY SOIL
Jodhpur
BLACK COTTON SOIL
Neemuch
SUB TROPICAL
Mehsana
CLAY SOIL
23°N
Ahmedabad

Map showing the range and distribution of different forms of *Withania somnifera* in India.

Figure 3

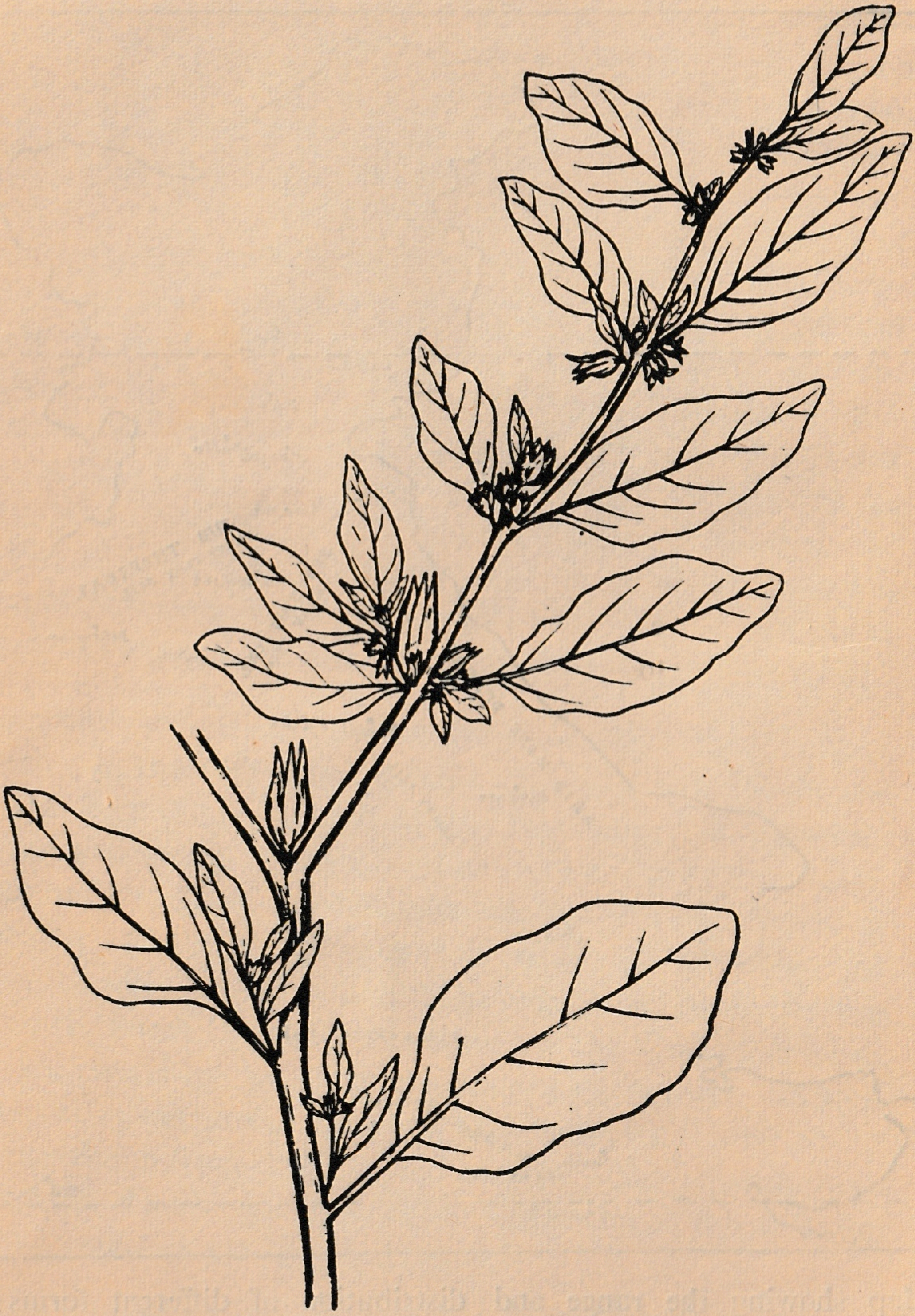


Illustration of Form 1 of *Withania somnifera* many; leaves small and densely pubescent, subcoriaceous, with distinctly undulate margin; the aerial shoots start branching a few cm. above the ground, branching lax, nodes and internodes compact calyx with partite incisions; corolla small. 2-3 mm, rotate companulate, lobes straight or slightly reflexed; fruits small enclosed in a moderately inflated, but tapering and elongate calyx, fruiting calyx moderately inflated, faintly ribbed, lobes deeply incised; berry red on some plants, straw-coloured on others; root straight tap, slender, unbranched or slightly branched, smooth, light coloured and starchy. Grown as an annual crop (Fig. 3).

Form II: This form is seen growing in the sandy desert soil of Pilani, Marwar and some other parts of Rajasthan. Plants are 0.6-1.5 m long; stem is single erect, grown from the crown which gives off branches above the ground level; leaves sharply acute, thin, membranous, flowering very lax, usually 1-2 flowers at a node; calyx lobes linear, deeply incised; corolla rotate, lobes same as calyx; fruiting calyx moderately inflated, faintly ribbed and ovato-oblong, lobes linear recurved; roots straight, unbranched, thick, fleshy and ditsinctly fusiform; root bark very light coloured and xylem portion soft and fleshy. (Fig. 4).

Form III: This is seen growing in Chandigarh and some other mountainous areas of Punjab and Uttar Pradesh. Plants 0.6-1 m tall; branching 15-30 cms above ground level; vegetative leaves very large, 7-15 cm., leaves on floral shoots 2.5-7.5 cm., densely pubescent, almost subcoriaceous, acute flowers 3-5 at a node;calyx and corolla campaunlate with straight obtuse, lobes not preading but recurved; fruiting calyx highly inflated, largest of ll the types, distinctly ribbed; berries larger than all other types oot a straight unbranched tap root, tapering gradually from crown ownward, comparable in size and texture to Form II but not fusiorm (Fig. 5).

Form IV : This is found growing near Delhi. Plants 0.6-0.75m ll, branching profusely near the ground; leaves small, vegeta-

Figure 4

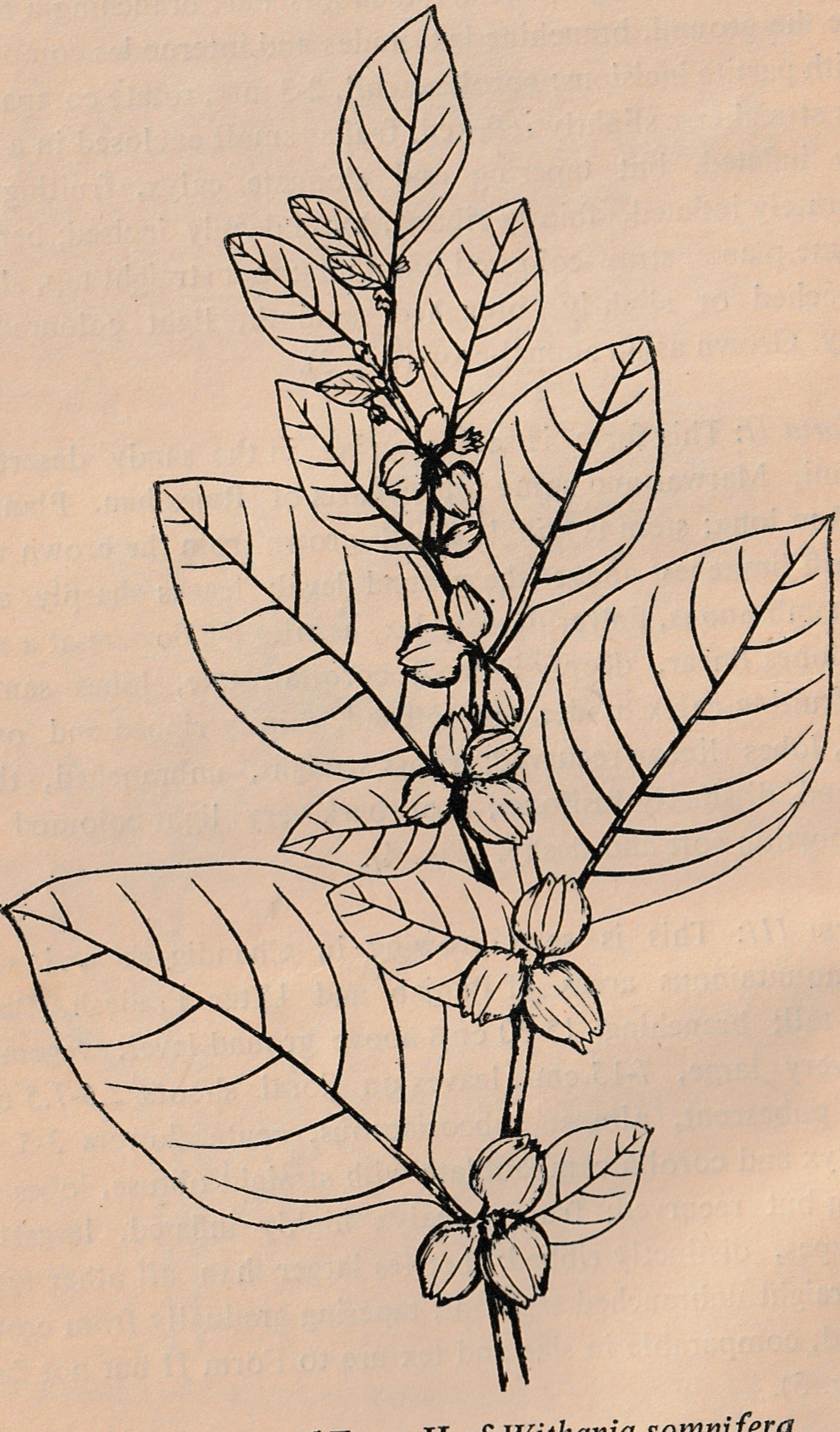


Illustration of Form II of *Withania somnifera*

Figure 5

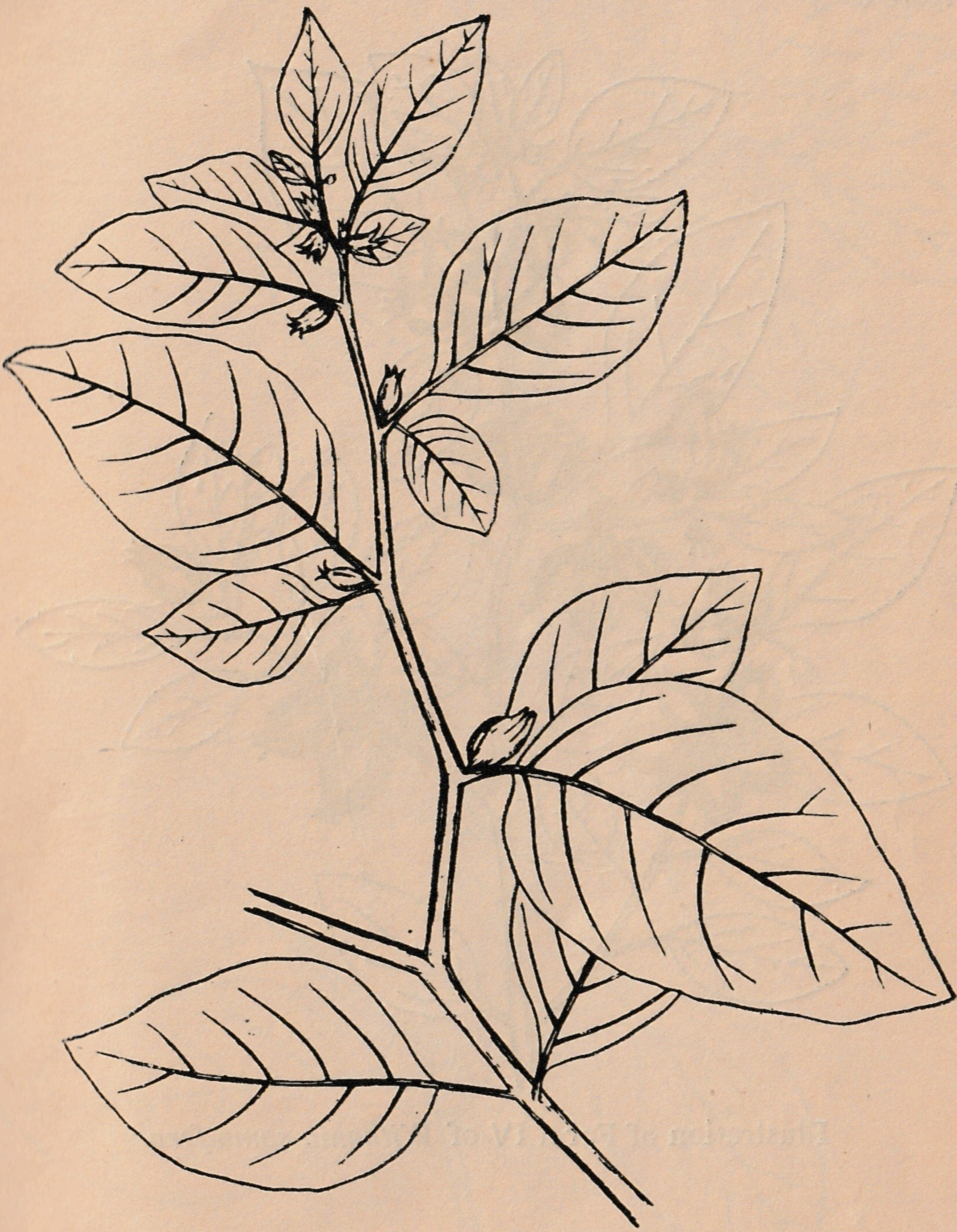


Illustration of Form III of *Withania somnifera*

Figure 6

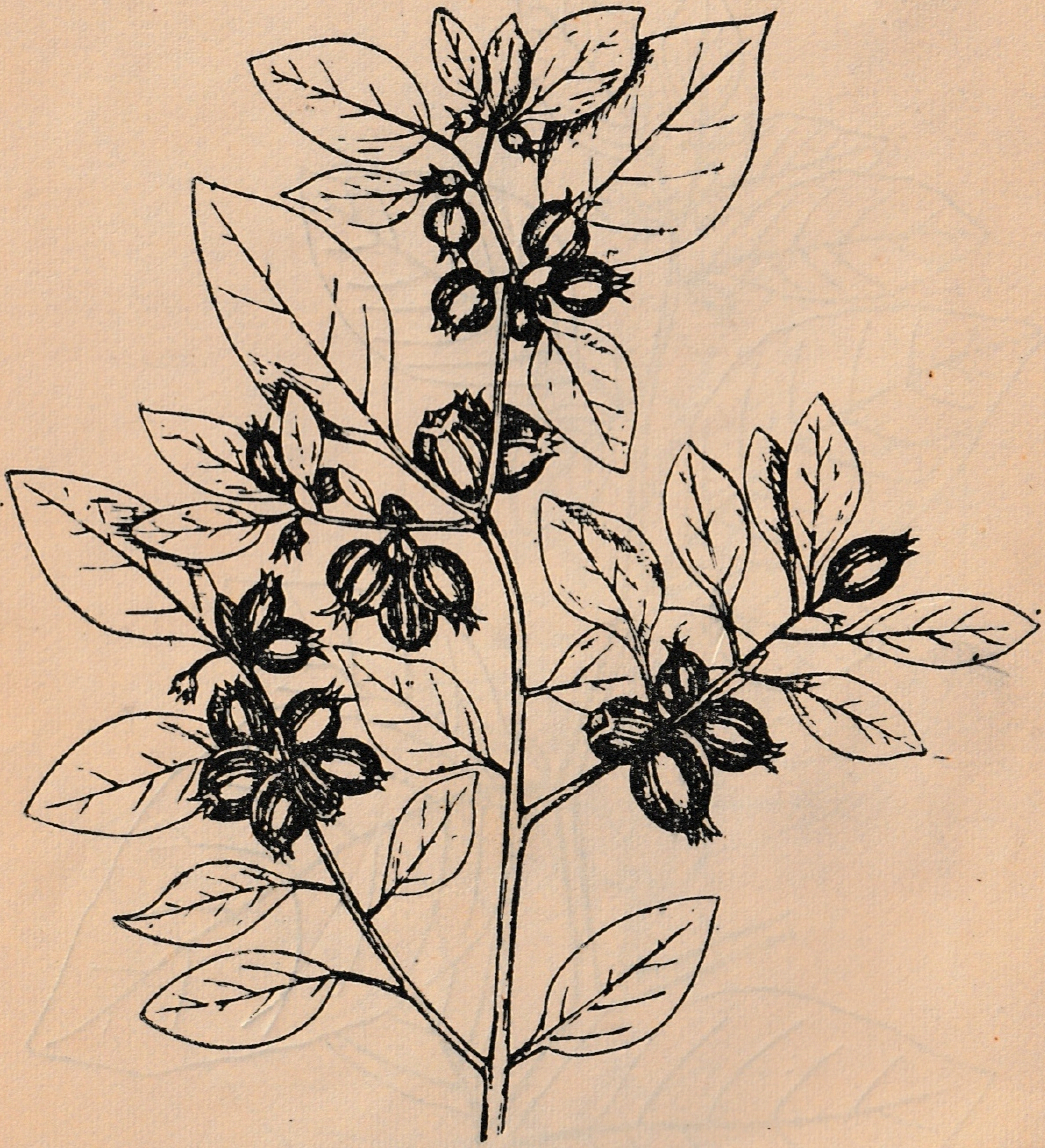
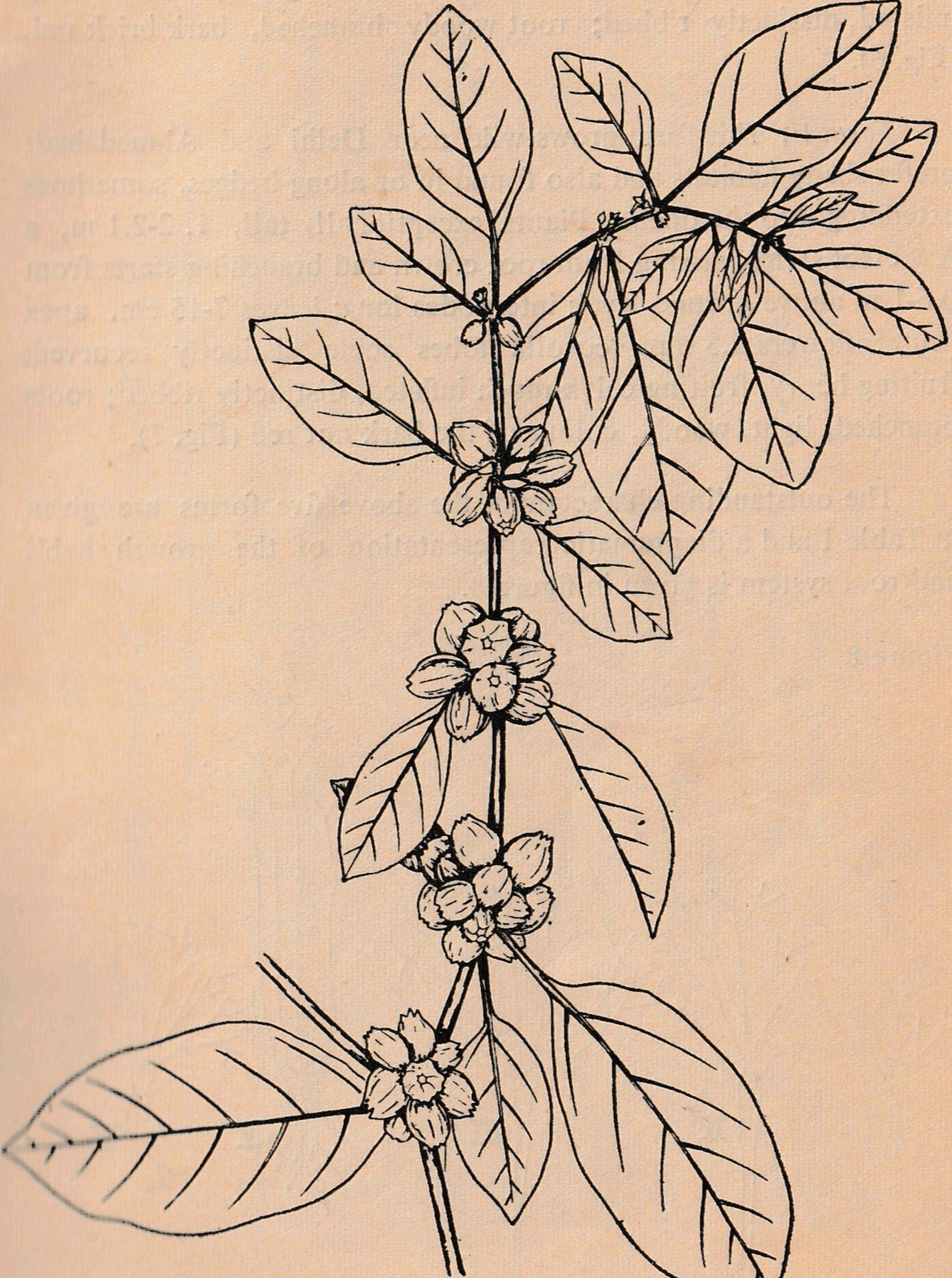


Illustration of Form IV of *Withania somnifera*

Figure 7

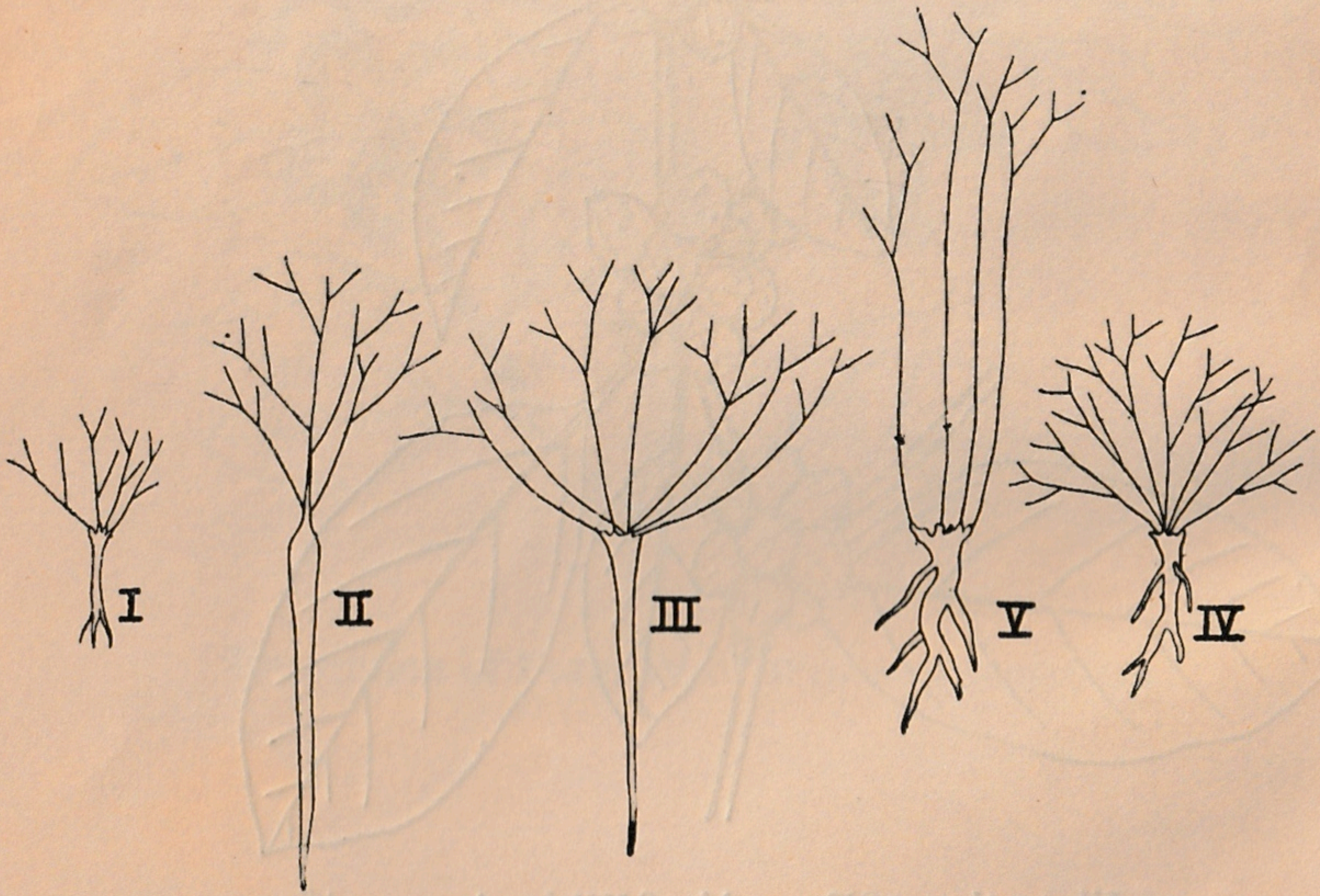


Illutration of Form V of *Withania somnifera* tive 5-7.5 cm. floral 2.5-3.8 cm.; flowering and fruiting heavy, flowers in dense clusters at each node; fruiting calyx moderately inflated, distinctly ribbed; root woody branched, bark brick red. (Fig. 6).

Form V; This form grows wild near Delhi and Ahmedabad; prefer shady habitats and also found in or along hedges, sometimes extending into the open. Plants exceptionally tall, 1. 2-2.1 m, a few erect stems, arising from root crown and branching starts from 0.6-1 m above ground level; internodes long; leaves 7-15 cm, apex acute; flowers 2.3 mm, corolla lobes acute distinctly recurved; fruiting heavy, fruiting calyx much inflated, distinctly ribbed; roots branched, light, woody, colour of the bark not red (Fig. 7).

The outstanding characters of the above five forms are given in Table I and a diagramatic representation of the growth habit and root system is given in figure 8.

Figure 8



Illustrations showing growth habit of forms 1 to V of *Withania somnifera*

TABLE 1

Characteristics of forms of *Withania somnifera*

	1	2	3	4	5
Root	Unbranched or slightly branching in lower half	Unbranched, erect	Unbranched, erect	Prominent lateral branches	Prominent lateral branches.
Texture	Soft, tuberous starchy	Very soft, tuberous	Soft tuberous	Hard, woody	Hard, woody
Crown	Slightly fusiform	Distinctly fusiform	Knotty, conic-tapering.	Knotty	Knotty
Colour	Very light, pale, brown	Light, pale brown	Light, pale brown.	Reddish brown	Dirty brown.
Bark	Smooth, thin pale brown	Very smooth thin, pale brown	Smooth thick, pale brown	Coarse, thick, brick red	Coarse thick, dirty brown

(Contd. from page 15)

Stem					
Height	Rarely taller than than 30 cm.	0. 6-1.2 m	0. 6-1 m	0. 6-0.9 m	1.2-2.1 m
No. of shoots from crown	One to many	One or few erect	Numerous procumbent	Numerous spreading	Few, erect.
Branching	Lax	Lax	Heavy	Extremely heavy	Lax
Leaf					
Apex	Obtuse tapering	Sharply acute	Sharply acute	Acute	Acute
Margin	Wavy	Entire	Entire	Entire	Entire
Thickness	Subcorianous	Membranous	Subcoriaceous	Moderate	Moderate
Hairyness	Dense	Slight	Dense	Moderate	Moderate
Veins	Inconspicuous	Faint	Faint	Faint	Faint
Arrangement on floral sheets	More than 2 at a node	2 at a node	2 at a node	2 at a node or irregular	2 or more at a node.

(Contd. from page 16)

Flower					
Calyx incision	Partite	Partite	Fid	Fid	Partite
Apex lobe	Acute	Sharply acute	Acute	Acute slender	Acute
Corolla shape	Rotate companulate	Rotate	Companulate	Rotate	Rotate
Apex lobe	Straight	—	Recurved	—	Recurved
Fruit					
Density	Up to 15 at a node	1-2 at a node	5-7 at a node	5-10 at a node	Up to 15 at a node
Fruiting	Moderately	Moderately	Highly	Moderately	Much inflated
Calyx	Inflated	Inflated	Inflated	Inflated	
Ribs	Faintly ribbed	Faintly ribbed	Distinctly ribbed	Distinctly ribbed	Distinctly ribbed

From the foregoing account it is inferred that the various forms of *Ashwagandha* may be different varieties of the same species, since intraspecific variations and polymorphism are usual phenomenon in Solanaceae, e. g. numerous forms of *Capsicum*[61], *Lycoperiscum esculentum* and *Solanum melongena* which do not possesss species rank. These authors conclude that there is an extreme degree of variability in *W. somnifera* with respect to growth habit and morphological characteristics of plants in different parts of India and in plants from other countries. In their opinion either the species is extremely polymorphic, or the name *W. somnifera* Dunal has been indiscriminately applied to wide variety of dissimilar forms, some of which at least deserve an independent rank as subspecies or varieties. The authors further state that for technical reasons, the name *W. ashwagandha*, suggested for cultivated plants, is not to be considered taxonomicaly valid. They stress the need for critical cytogentic and ecological studies in order to assign proper taxonomic status to the various forms. Anatomical differences in the roots of the forms have also been reported and are described in the subsequent pages.

Cultivation, collection and marketing of *Ashwagandha*

Atal and Schwarting[5] made a survey regarding its cultivation, colleciton and marketing of this drug. They observed that only a very small collection of roots of *W. somnifera* is obtained from plants growing wild in Bikaner and Pilani areas. The bulk supplies of *Ashwagandha* roots are obtained exclusively from cultivated sources, the Manasa plantations in Madhya Predesh. It is believed that the Manasa plantations originiated from the supplies of the drug form Nagore, a few hundred miles away in the adjoining State of Rajsthan. The drug from Manasa is, therefore, sometimes called and sold under the name "*Ashwagandha Nagori*". These days the fields of *Ashwagandha* occur in radius of eight miles, with Manasa as the centre, covering a total area of about 4000 acres. Sometimes when there is an increased demand for the drug, the cultivation is extended to an area 14 miles around Manasa.

A limited cultivation of *Ashwagandha* is undertaken near Arnod in the district of Partapgarh. The cultivation of *Ashwagandha* does not require much attention and labour. The crop is grown on soil which is unsuited for other crops.

The seeds are sown in June or July before the onset of rainy season. On average 5 pounds of seed is sown per acre but the amount is increased in rich soil and decreased in case of poor soil. Sowing is done by broadcasting. No special preparation or cure of the land is required to plant this crop. Addition of nitrogenous fertilisers such as ammonium sulphate produces plants with heavy leaf growth and small roots. No irrigation is given throughout the growing period. Excessive rain fall is harmful because it leads to extensive weed growth. The plants flower and fruit in December and January; harvesting is started in Jannary and continued till late March.

The whole plant is uprooted for the collection of roots. The roots are separated from the overground parts as soon as possible. The whole root system is either dried as such or by cutting into smaller pieces. The yield of dry roots per acre ranges from 150 pounds to 300 pounds. The Manasa area produces about 670,000 pounds of dry roots annually and in some years when the demand is more the production may be as such as three times of the normal production. The whole trading of this drug is done in Manasa. The roots either in whole dried condition or transversely cut dried pieces are subjected to further cleaning, trimming, and grading before they are despatched to various drug markets in India.

The main tap root is cut into transverse pieces 4-8 cm long. These transversely cut roots are hand sorted into different grades. This grading is done keeping in view the diameter, uniformity in size, external surface, colour of the pieces. The superior grade is one with 1-1.25 cm. diameter, smooth external surface, light colour and completely devoid of any root crown. There are extreme variations in the price ranges of different grades; the price range being Rs. 5.00 to 70.00 per 82 pounds but this is subjected to variation of the total production at Manasa. About 50% of the production is sent to Bombay, 20% to Calcutta, another 20% to Delhi and remaimg to other less important markets.

PHARMACOGNOSTIC STUDIES

Pharmacognostic studies

Although quite a number of papers have been published on historical studies of *Withania somnifera* (Linn) Dunal, yet the results of differnet authors do not tally with each other.

Prasad et al[58] who studied the pharmacognosy of *Withania somnifera* collected from the botanical garden of the Ayurvedic College of Banaras Hindu University, Banaras and the commercial sample procured form the market did not report any differences. Shah et al[66] who studied the phamacognosy of drug of commerce and samples obtained from Central Drug Laboratory, Calcutta, reported tuberous roots. This appears to be erroneous as the *Ashwagandha* plant possesses tap root only. Dutta and Mukerji[12], I.P.[56] mentioned absence of crystals. Neuwald and Lodges[54] observed dark granular masses in parenchymatous cells. Shah et al[66] reported that these dark masses are insoluble in acetic acid but soluble in HCl and 75% H_2SO_4 forms acicular crystals, and concluded that the dark masses are identifiable as Calcium oxalate. Dhalla et al[10] also studied the pharmacognosy of *W. somnifera* in wild and the cultivated plant of *W. somnifera* so called *W. ashwagandha* Kaul. They observed the following difference in these two varieties (Table 2).

Atal and Schwarting[4] also reported the pharmacognostical differences in the three samples of *W. somnifera* obtained from different sources. They studied the market sample, the sample of the authentic plants growing near Delhi and Chandigarh and the roots of the plant grown in the drug garden of the University of Connecticut, (U. S. A). They noted the significant differences in the roots of three sources.

They reported that "the cork of the commercial sample is thin 2-3 cells wide and the cells measure 34-60μ tangentially and 24-40μ-

TABLE 2

Differences between *W. somnifera* **&** *W. ashwagandha*

	W. somnifera	*W. ashwagandha*
Roots	Earthy brown in colour, not uniform in appearance and break with an irregular fracture.	Yellowish brown in colour. uniform in appearance and break with an even fracture.
Rind	Easily separable, cannot be easily powdered.	Not separable, easily powdered.
Root hairs	Present	Few
Starch grain	Very few	Starch grains are present in abundance in almost all the cells of the paranchyma, right from the cork up to central steel.
Central steel	Central steel forms a very compact cylinder of xylem in which the vessels are not so regularly arranged.	Central steel are loosely arranged and consist of patches of vessels which are broader towards the periphery and narrower towards the central part.
Size of xylem	Small	Big

(Contd. from page 24)

Ash analysis (total ash, acid insoluble ash and constituent)	5.25%, 1.23%, chloride, sulphate, phosphate, Na, K, Fe, Ca and Mg.	6.48%, 1.65%, chloride, sulphate, phosphate, Na, K, Fe and Ca.
Percent yield of successive extracts (Pet. ether, 40°-60° ether, chlorofom, alcohol).	0.65, 0.86, 0.54, 3.2	0.45, 0.2, 0.21, 4.19
Contents of inorganic salt mixture isolated	Na, K, chloride, sulphate, and phosphates.	Na, K, chloride, nitrate, phosphate and sulphate.

(Contd. from page 25)

Presence of various chemical ingredients	Alkaloids, glycosides, sterols, resins, reducing sugars, tannins and inorganic salts.	Alkaloids, glycosides, sterols, resins, reducing sugars, starch and inorganic salts.
Amino acid present	Cystine, glycine, glutamic acid, valine, tyrosine, proline, -alanine and tryptophane.	Cystine, aspartic acid, serine, glutamic acid, valine, proline β-alanine typtophane and phenylalanine.
Colour of Dragendorff's precipitate of the total alkaloid	Dirty reddish brown.	Bright reddish-orange.
Total alkaloids (yield, number of bands, Rf value).	0. 14%, 6,0.34, 0.52, 0.62, 0.66, 0.72, & 0.82	0.20%, 7, 0.28, 0.48, 0.52, 0.60, 0.64 0.70 and 0.90.

radially. The cork of the authentic root is broad 3-10 layers, lignified and the cells are collapsed and show a poor radial arrangement. The secondary cortex in each is similar showing starch 2-3 compound, 5-30 μ in diameter, with a characteristic dot shaped, linear or cross shaped, central hilum. A few celles are filled with microsphenoidal crystals of Calcium oxalate. The phloem in each is similar.

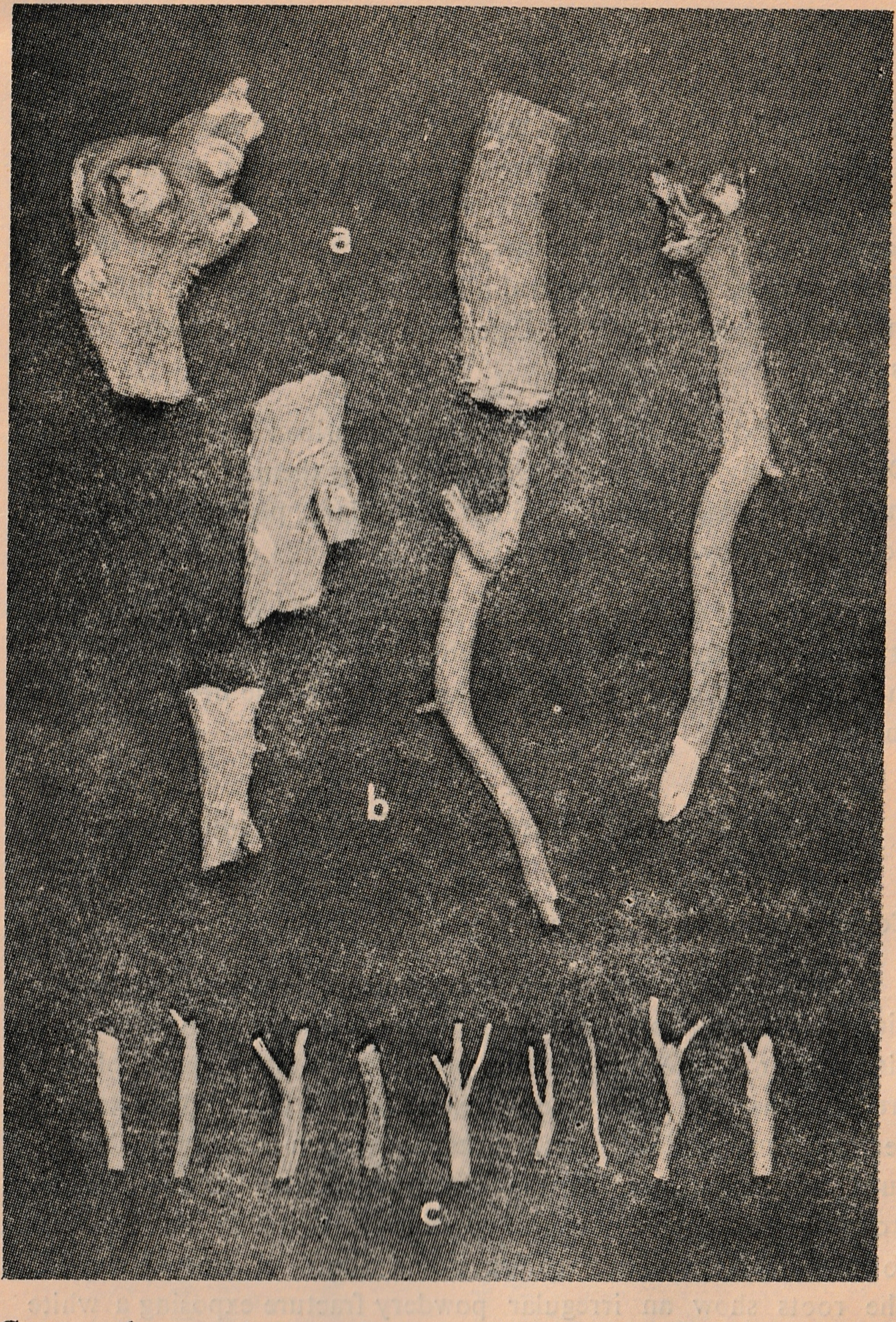
They did not find latex vessels as described by Neuwald[54] and Dutta[12] in any of the specimens examined by them. The presencefor microsphenoidal crystals and the intraxylary phloem, were also not observed by these authors.

In view of the contradictory reports on the pharmacognostical studies of the drug by different authors, a systematic morphological study of the various forms of the drug plants was carried out.

Morophological Characters of the various forms

Commercial Sample : (Figure 9c)

These roots occur as pale brown to pale white in transverse pieces, 3-8 cm. long and 4-10 cm. in diameter. Some of these pieces bear a root crown and occasionally the remnants of pale green stems, the latter rarely exceeding 4 mm in diameter and bearing alternate leaf scars. The root crown shows a nunber of bud scars. The part of the root immediately below the root crown is constricted and longitudinally wrinkled but further downwards it gradually enlarges and shows a smooth surface. The roots show an irregular powdery fracture exposing a white surface. Treatment of the fractured surface with phloroglucinol reagent shows a thin bark occupying one-fifth of the total diameter of the root. The stained xylem elements form an interrupted ring immediately inside cambium and small scattered groups in the large central white pith (Figure 10).



Comparative morphology of commercial *Ashwagandha* and roots of plants collecten from wild.

a) thick and light textured roots characteristic of plants from Chandigarh and Pilani.

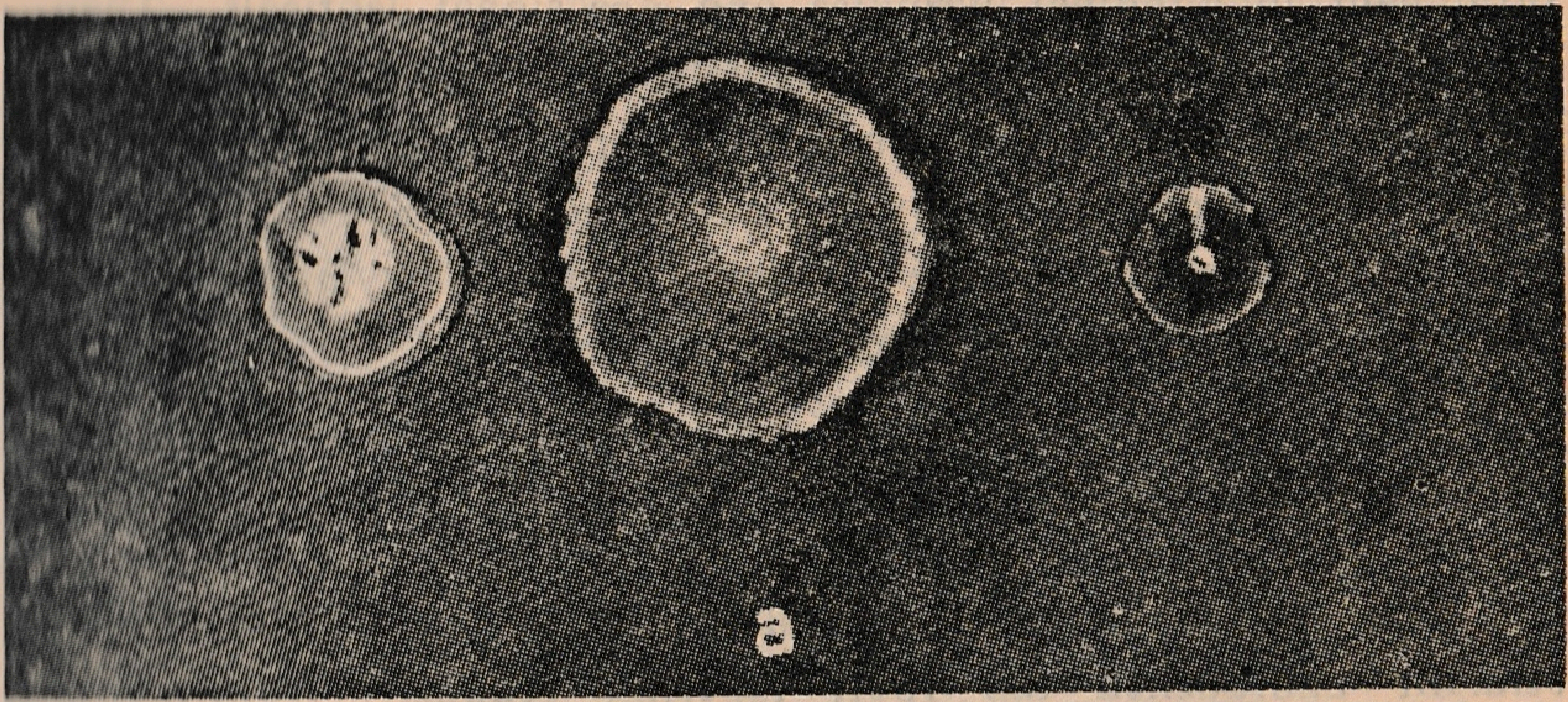
b) Compact woody roots characteristic of plants growing in clayey soil of plains like Amritsar and Delhi.

c) Commercial roots which are much smaller in size and are white and starchy.

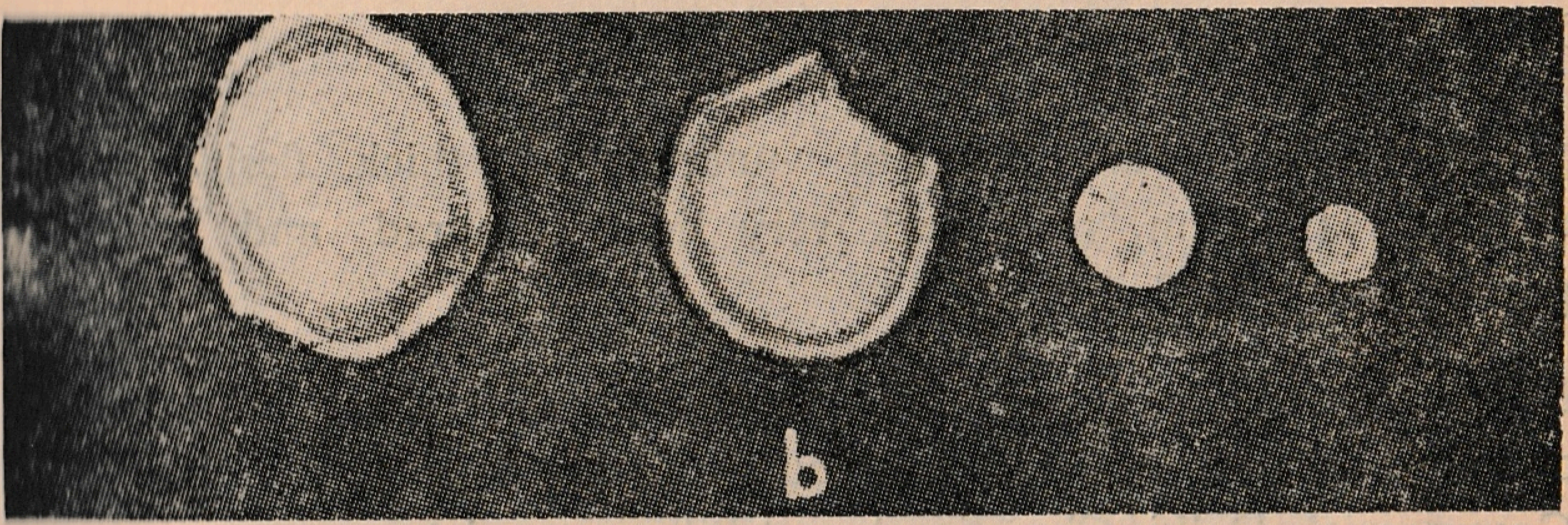
Figure 9

Figure 10

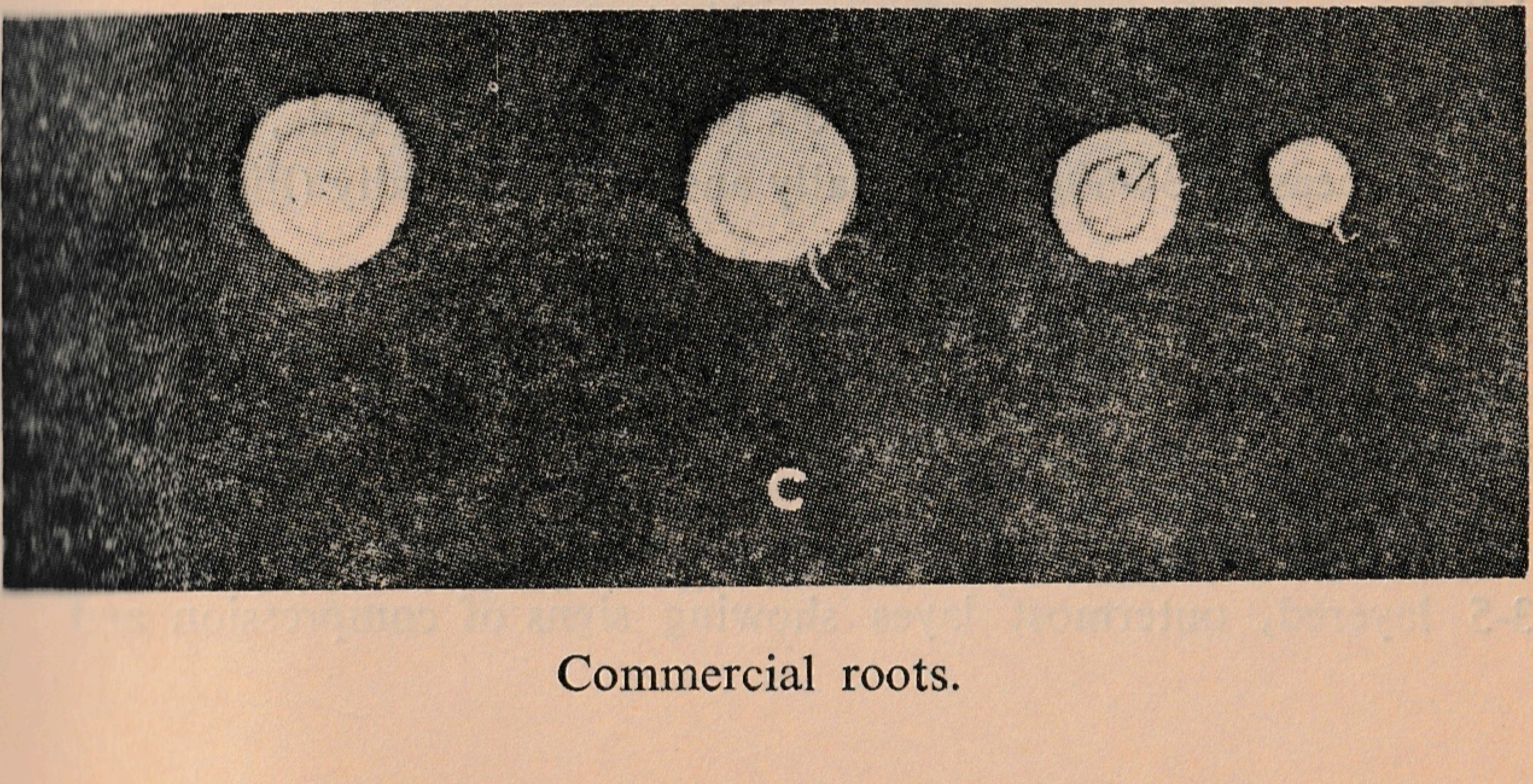
Comparative appearence of commercial *Ashwagandha* and roots from the wild; the roots have been cut transversely and stained with phloroglucinol to emphasise the distribution of lignified elements.



Woody roots of Amritsar and Delhi type



Semi woody roots of Chandigarh and Pilani



Commercial roots.

Delhi Sample : (Figure 9)

The roots are brown to reddish brown in colour and show prominent longitndinal wrinkles and occasional lenticels. They are many times in length and diameter compared to commercial roots and bear no resemblance in external appearance to the latter. The roots invariably had a solid core of xylem. Only a few roots showed variable scattered groups of xylem which is characteristic of roots of commerce.

Chandigarh Sample : (Figure 9a)

The root collected from plant growing at Chandigarh is straight unbranched tap root, tapering gradually from crown downward, thick, fleshy, soft and pale brown.

Pilani Sample (Figure 9a)

The roots are straight unbranched, thick fleshy and distinctly fusiform, root bark very light coloured and xylem portion soft and fleshy.

Microscopical Character : The significant differences have been observed in the structure of roots of the four forms (i. e. Commercial, Delhi, Chandigarh and Pilani sample).

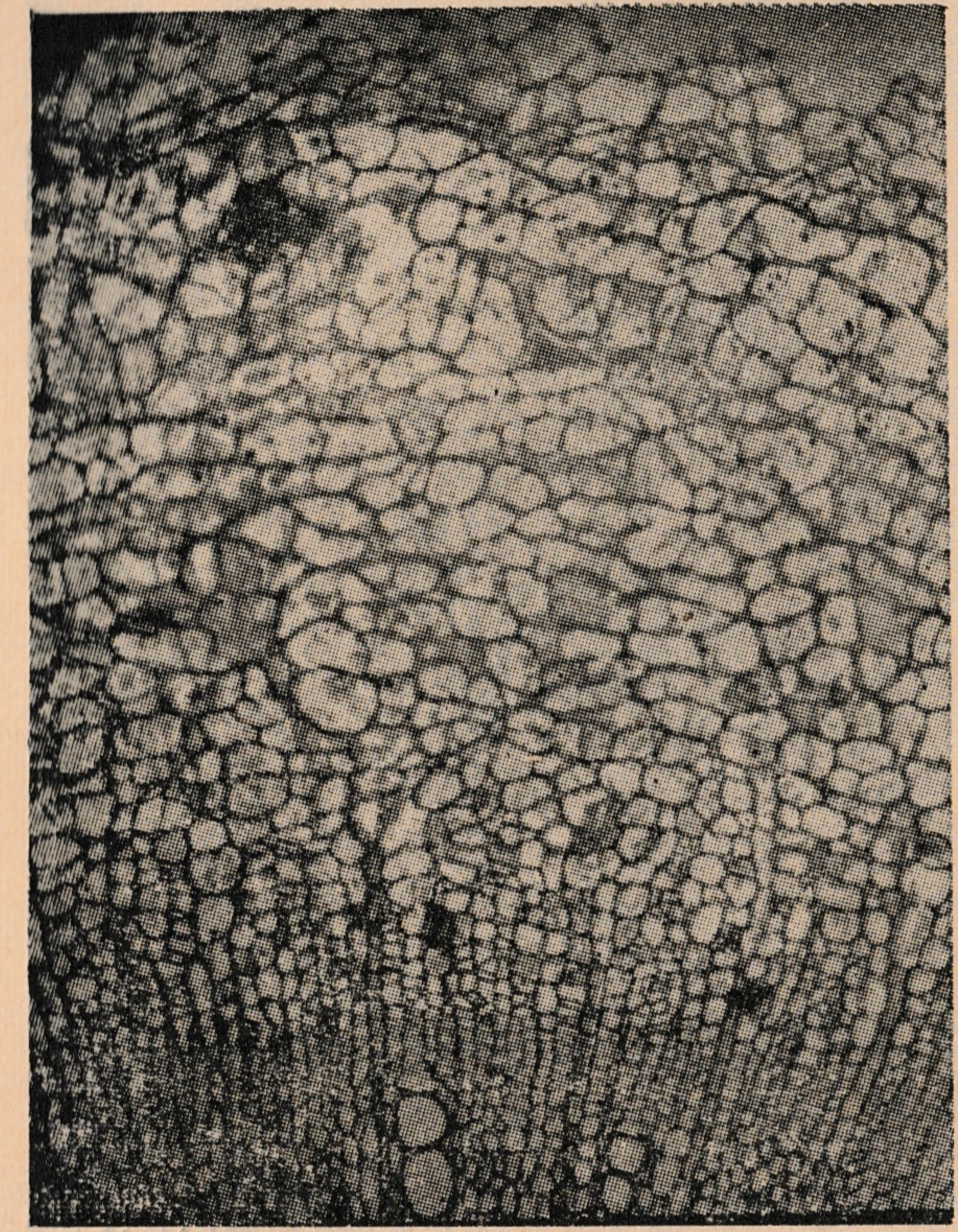
Cork :

The cork of commercial roots is thin, 2-3 cells wide, and the cells measure 34-60μ tangentially and 24-40μ radially (Figure 11).

The cork of the roots of Delhi sample is broad, 3-10 layers lignified. The cells are collapsed and show a poor radial arrangement (Figure 12 & 13).

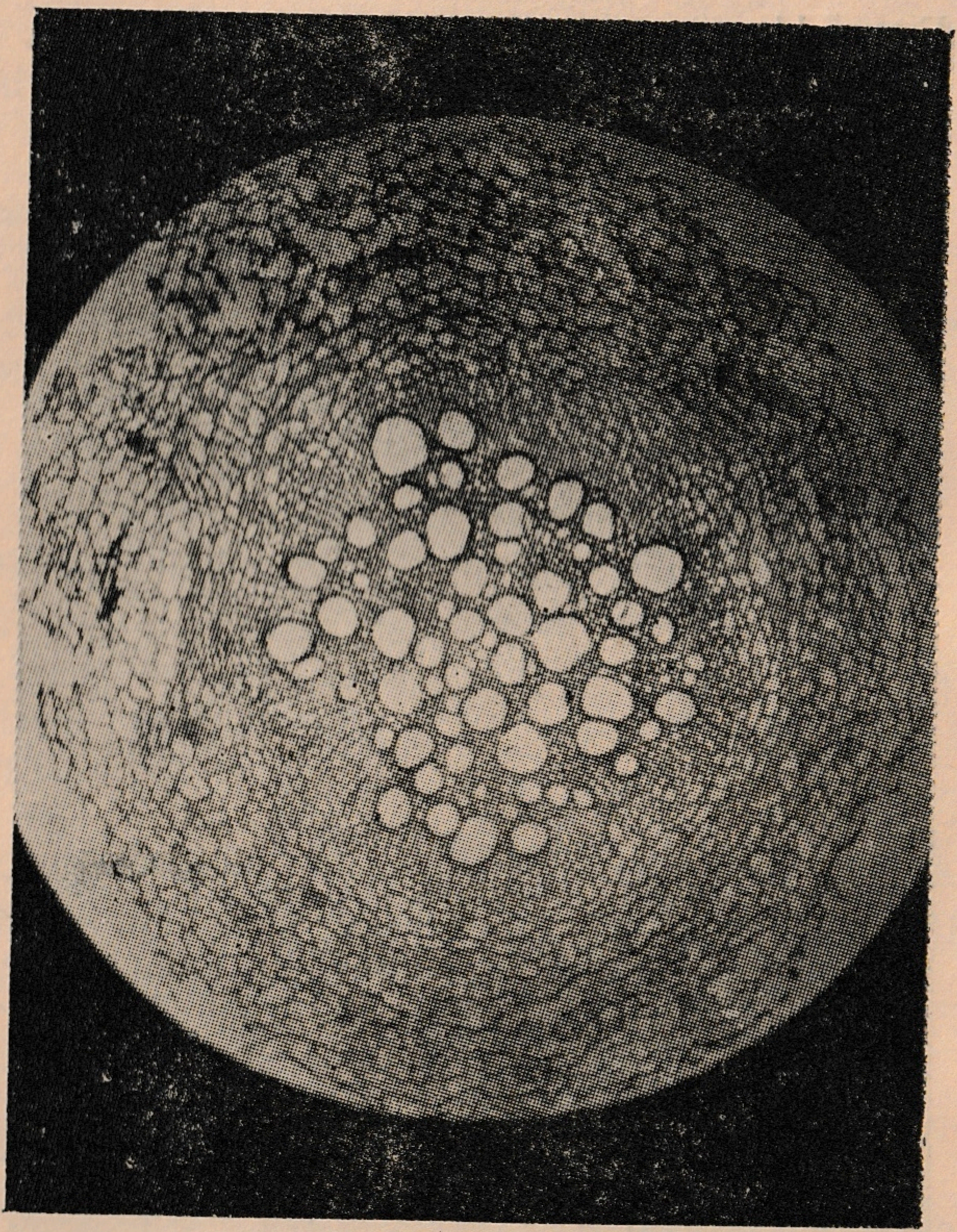
In the case of Chandigarh roots, the cork is medium sized, 3-5 layered, outermost layes showing signs of compression and

Figure 11



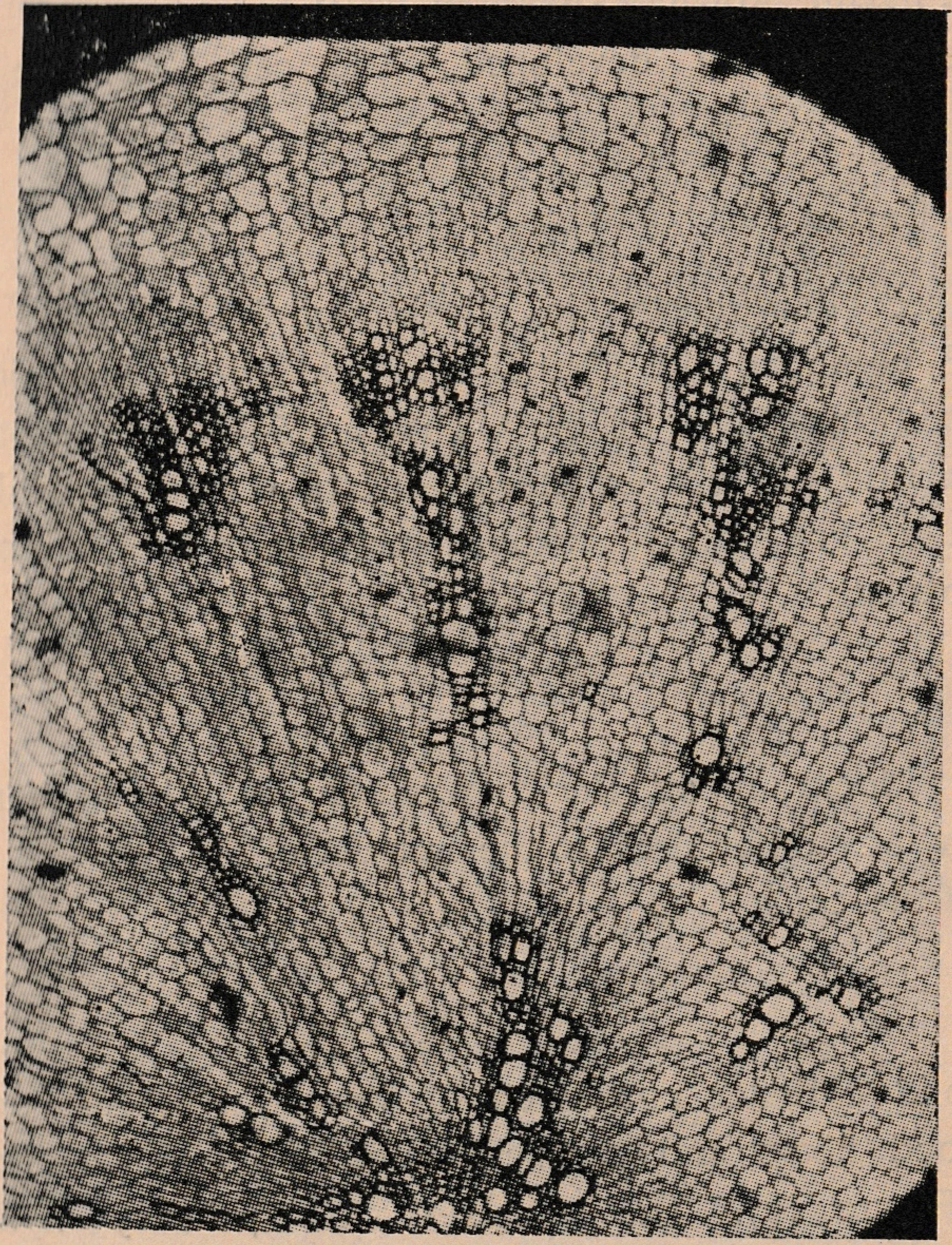
T. S. of cork, cortex, phloem and a part of xylem region of commercial *Ashwagandha*.

Figure 12



T. S. of a very thin lateral root of Delhi type. Note the solid lignified core with large sized vessels.

Figure 13



T. S. of a young root (main axis of Delhi type) showing development of much lignified element in contrast to commercial roots.

disintegration. The cells of inner layers are irregular and do not show the usual radial arrangements. These cells measure 39-55μ tangentially and 15-30μ radially.

The cork in the Pilani sample is thin only 2-3 layers broad, the inner layers consisting of small rectangular cells, tangentially elongated, having brownish suberised walls and are closely packed. The tissue is poorly developed. The cells measure 16-66μ radially.

Secondary Cortex :

The secondary cortex in all the samples is similar, showing starch 2-3 compond (in Chandigarh, and Pilani samples single starch grain may also be seen). They are 5-30μ in diameter, with a characteristic dot shaped linear or cross shaped central hilum. Some cells are filled with microsphenoidal crystals of calcium oxalate.

Phloem

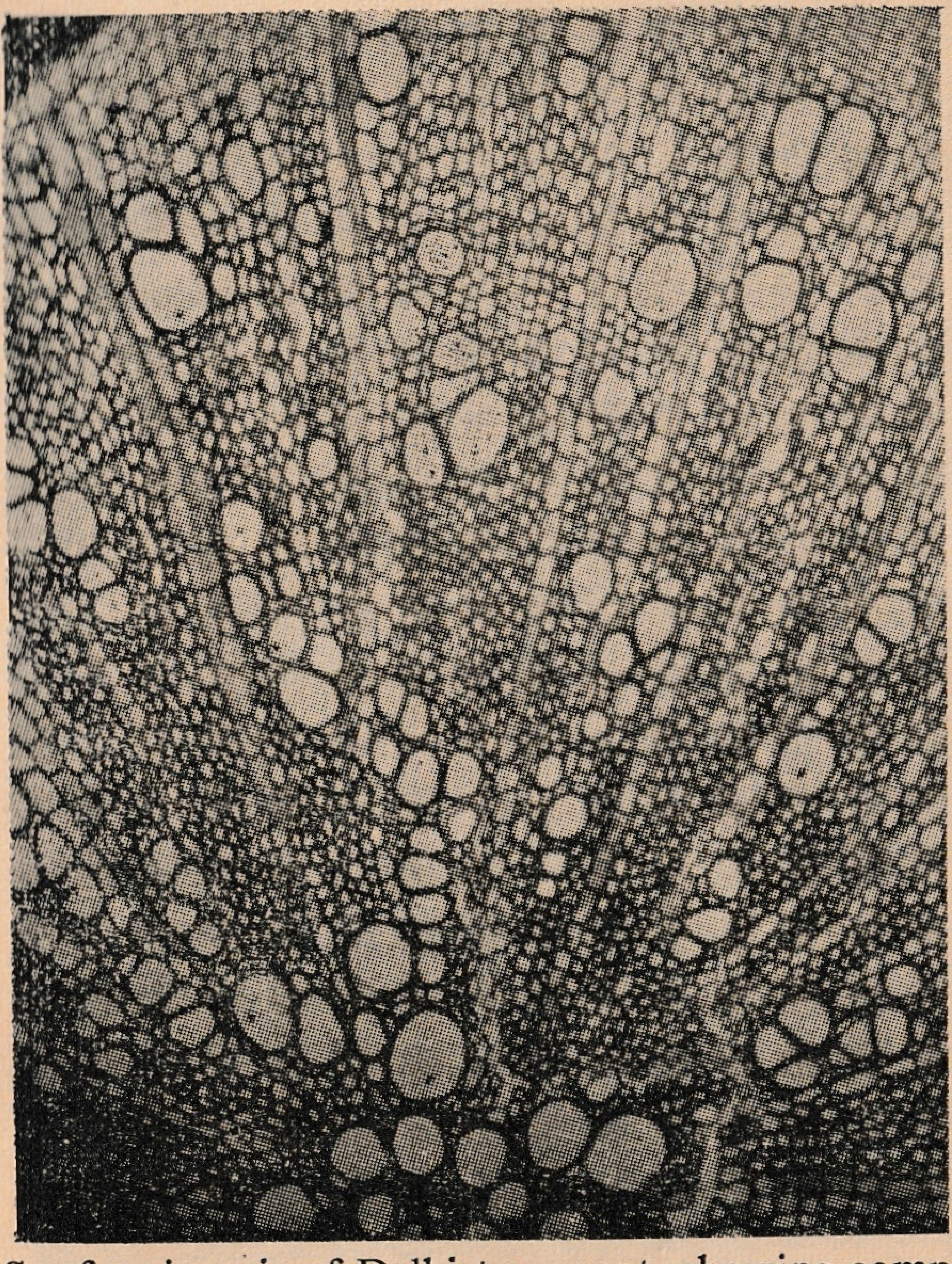
The phloem in all the samples appears to be similar except that there are differences in the number of the layers of cells or the size of the cells.

Xylem :

A solid cylinder composed of vessels, tracheids, tracheidal fibers, wood fibers and parenchyma of the lateral roots of Delhi sample (Figure 12). The vessels of the primary xylem are compactly arranged. Vessels measure 200-800μ in length and 75-225μ in breadth. The tracheids show pitted thickening and blunt end walls and are 225-375μ long and 30-50μ broad. Tracheidal fibers are septate and non septate. Fibers are 440-750μ long and possess oblique elliptical pits and have pointed or bifurcated ends. The xylem is eompleted lignified after secondary growth (Figure 14).

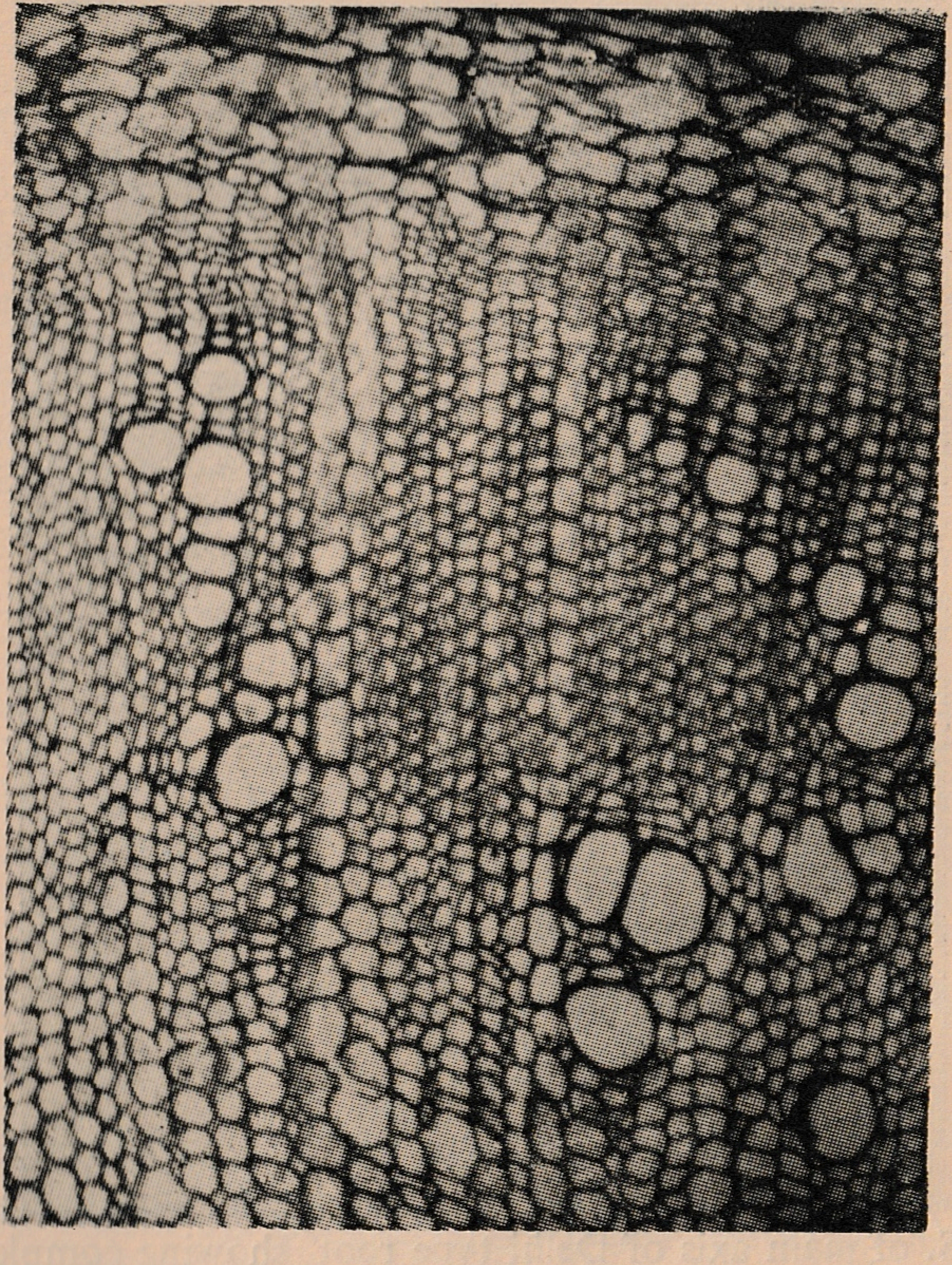
In the commercial roots (Figure 15 a. b, c) a discontinuous as well continuous cylinder were observed. Central

Figure 14



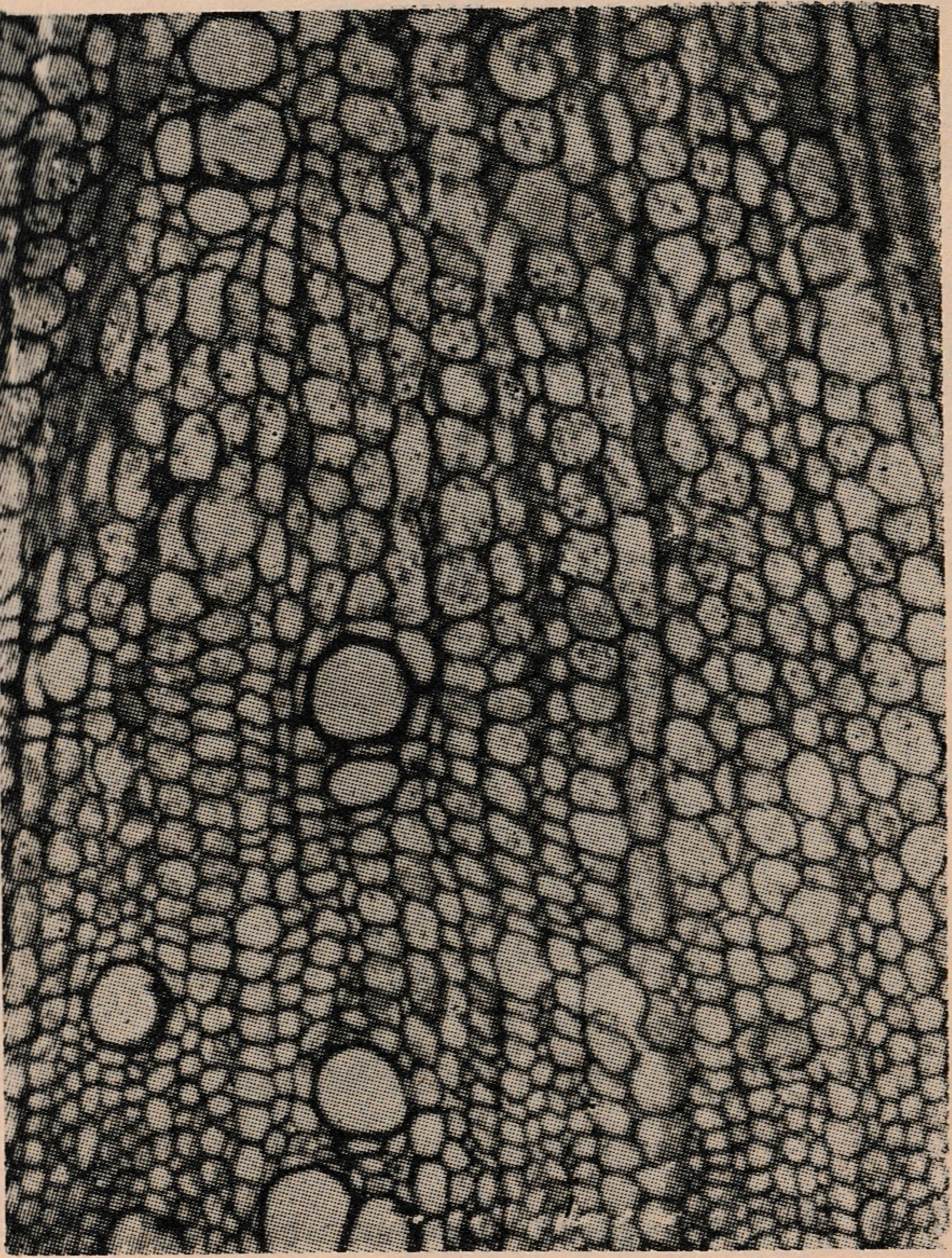
T. S. of main axis of Delhi type root showing complete lignification after secondary growth.

Figure 15 (a)



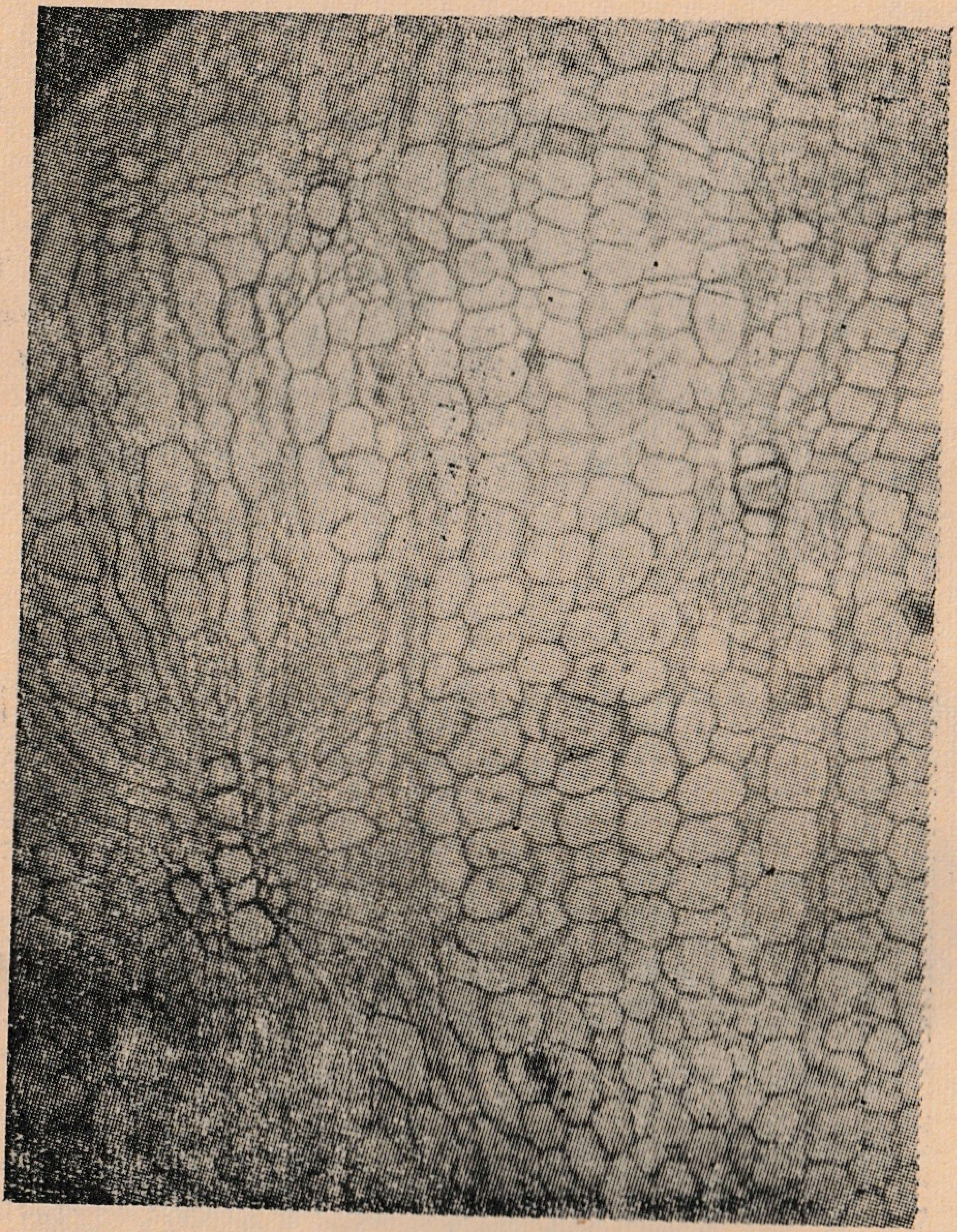
T. S. of xylem region of commercial root—
Outer xylem

Figure 15 (b)



T. S. of xylem region of commercial root—
Middle region of xylem

Figure 15 (c)



T. S. of xylem region of commercial root—Xylem near the central portion showing patches of intraxylary phloem.

parenchymatous matrix was present and xylem patches of 1-8 vessels as well as interxylary phloem patches are apparent. Primary xylem is present in the centre of this area. Vessels are 150-250μ long and 60-90μ broad. The tracheids and tracheidal fibers are 150-300μ long and 23-35μ broad. Fibers are 300-600μ long. Xylem parenchyma contains starch, 8-19μ in diameter and a few cells are filled with microspenoidal crystals of Calcium Oxalate (Figure 16).

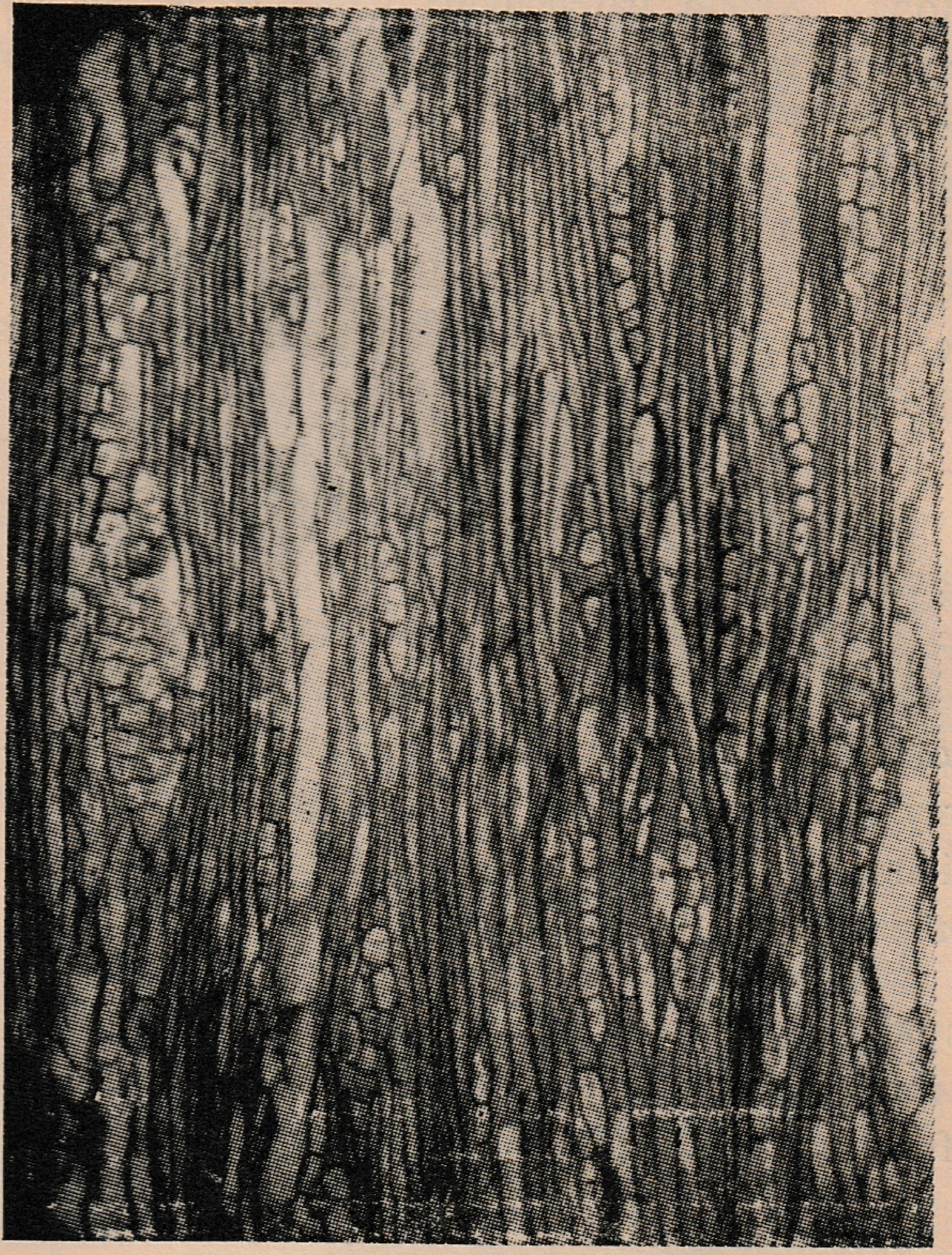
In Chandigarh sample, xylem vessels occur in groups of 2-6 vessels associated with tracheids and fibers and are aggregated more near the cambium line. There are about 21 vessels per sq. mm. in the cambial region of xylem while in the main central region there are about 10 vessels. Only in the Pilani sample the number of vessels in each group is 2-3. They are also aggregated more along the cambium line being about 6 vessels per sq. mm. in areas close to cambium while 5 vessels per sq. mm. in the main region of xylem. In Chandigarh sample, the primary xylem is composed of xylem vessels, tracheids and fibers and is intercepted by medullray rays or a matrix of non lignified parenchymatous cells. In Pilani sample the primary xylem is less developed but forms a compact mass of small vessels.

The study shows that the roots of commercial drug differs from the roots collected from wild plant, but resemble the roots collected from cultivated plants.

Estimation of total alkaloids of *Ashwagandha*

The method of assay proposed by Srivastava and Krishnamoorthy[69] determines, the chloroform soluble alkaloids only. Srivastava, Singh and Iyer[70] proposed a method for standardisation of the total alkaloids present in the extract based on the precipitation of the alkaloids present as reineckate salts, decomposition by sodium hydroxide and estimation of the thiocyanate thus liberated with mercuric nitrate. Srivastava[71]

Figure 16



Tangential longitudinal section of the xylem of commercial root.

has determined the total alkaloids by a gravimetric method by precipitation with Dragendorff's reagent and decomposing the precipitate with silver carbonate. The estimation of five alkaloids obtained by simulated biosynthesis by El-olemy and Schwarting[55] has been accomplished by TLC.

It was found that there is a straight line relationship between weight and area of each of the five alkaloids.

The above methods are lengthy and involve many steps. Moza and Singh[53] have reported a simple method based on non-aqueous titration for the estimation of total alkaloids of *Ashwagandha*. The method involves the titration of alkaloid mlxture (purified by passing over alumina) against standard perchloric acid using methyl violet as an indicator.

By this method, total alkaloid content of *W. somnifera* roots procured from wild plants of Chandigarh, "*Ashwagandha Nagori* of commerce and plants cultivated in Jammu gave the following results : Chandigarh variety 0.88%, commercial 1.09% and Jammu grown material 0. 68%.

Uses and pharmacological action of *Ashwagandha* :

Since the time of Charaka, *Ashwagandha* has been used for the cure of various diseases. In Indian system of a medicines, it is regarded as a very good sex stimulant and is also used mixed with other drugs for the cure of rheumatism, gout, skin diseases, nervous disorders, insanity, hypertension, ulcers and swellings. The leaves are supposed to reduce pus formation, inflammation and to promote healing processes. Fruits are also considered as diuretic.

Ashwagandha is used throughout Inda as a safe remedy, as a tonic in geriatrics, being efficacious in relieving hand and limb tremors of elderly people. It is prescribed for all kinds of weaknesses and is supposed to promote strength and vigour, and is regarded as aphrodisiac and rejuvenator. The roots are official in Indian Pharmacopoeia[56].

Modern pharmacological studies have revealed many interesting properties and uses to which this drug can be put after thorough investigation. Trebut[76] reported that the drug contained an alkaloid and possessed hypnotic properties. Power and Salway[57] however failed to record any marked physiological activity either in the whole drug or the isolated base, but Majumdar[46] reported that the major alkaloid withanine possessed a marked sedative and hypnotic action. Kapaczewski[26] has reported that the juice of the fruits possess milk coagulating properties and has also isolated a hypnotic fraction. Malhotra et al[47, 48] have shown that the total extract of the drug exhibits marked sedative action on mice, rats, rabbits, dogs and monkeys and a relaxant and antispasmodic action on ileal and tracheal muscles. The same authors have also shown this drug to potentiate the action of phenobarbital sodium and also that an extract of *Ashwagandha* has prolonged hypotensive, bradycardiac and respiratory stimulant actions.

Kurup[28] isolated an antibiotic principle frnm the leaves of *Withania somnifera* which exhibited marked activity against *Staphylococcus aureus*. Kurup[29] isolated another compound which inhibited the gram-positive bacteria and Ranikhet Virus. The inhibition of the antibacterial activity against *S. aureus* of the unsaturated lactone antibiotic from the leaves of *Withania somnifera* by cysteine and glutathione, the inhibition of glucose-6-phosphate dehydrogenase of *Bacillus subtilis* by the antibiotic substance and the mechanism of antibacterial action of antibiotic were also reported. [30, 54, 51].

CHEMICAL STUDIES

Chemical Constituents of *Withania somnifera*

It is because of the sedative and tranquilising properties of the roots of *Withania somnifera* (Linn.) Dunal. and the use of the plant for centuries in the treatment of numerous maladies by Ayurvedic physicians, that an increasing number of studies have been made of the constituents of *Withania somnifera.* in the last four decades. A large number of compounds have been isolated by various workers and the majority of these compounds belong to the group of alkaloids and steroidal lactones.

The first scientific investigation on the chemial principles of this plant appeared in the year I911, when Power and Salway [57] reported the presence of a number of compounds from the roots and leaves of *Withania somnifera*, namely, two new monohydric alcohols, withaniol, $C_{25} H_{33} O_4. OH$ and somnirol, $C_{32} H_{43} O_6.OH$; a new dihydric alcohol, somnitol, $C_{33} H_{44} O_5 (OH)_2$; an acidic hydrolytic product, withanic acid, $C_{29} H_{45} O_6.$ COOH, m.p. 226°; a nitrogen containing component, $C_{12}H_{16}N_2$, m. p. 116°; a phytosterol, $C_{27}H_{46}O$, m.p. 135-136°and ipuranol, $C_{25}H_{38}O_2(OH)_2$ besides a mixture of fatty acids, consisting of palmitic, stearic, cerotic, oleic and linoleic acids; an essential oil and sugar.

A little more than two decades later Majumdar and Guha[44] resumed the chemical study and reported the isolation of three amorphous bases from a Bengal variety of *Withania somnifera.* Further investigations of Majumdar [45,46] established the presence of several nitrogenous bases and partially charatcterized seven amorphous bases and also identified nicotine as the eighth component, namely, (1) somniferine, m. p. 185-187°; (2) somniferinine, m. p. 120°; (3) somnine, m. p. above 300°; (4) withanine m. p. 75-80° (5) Withananinine, m. p. 112-114°; (6) withananine m.p. 87-88°; (7) Pseudo-withanine, m.p. 155-156°. First six have been claimed to be new alkaloids and seventh one is the disintegrated product of withanine. However, no structural formula was reported for these substances by the author.

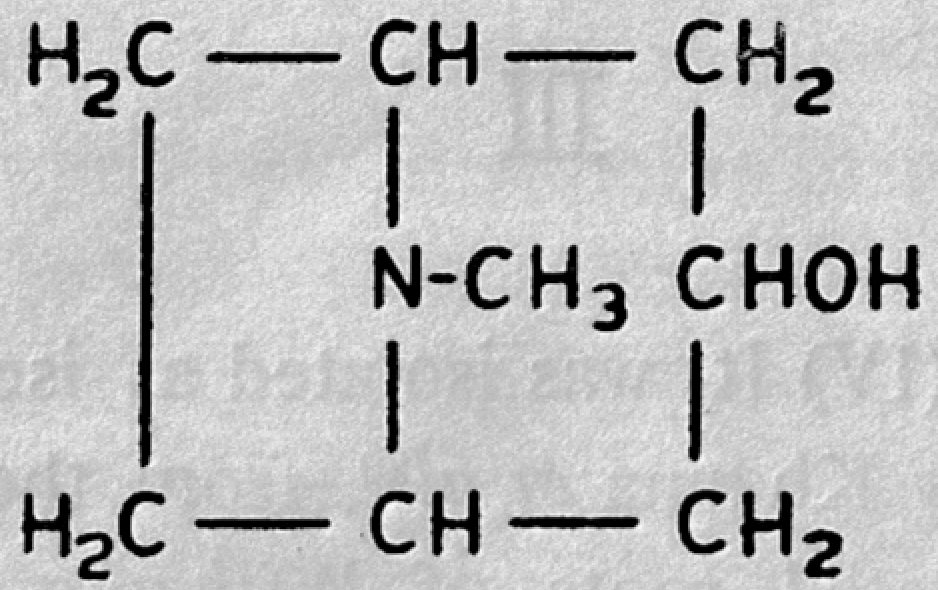
Out of these, the main alkaloid is withanine and comprises 38% of the total alkaloidal material. It was found to be chloroform additive compound having composition $C_{44}H_{80}N_2O_{12}$. $CHCl_{80}$. It gave on distillation with sodalime, pyridine, pyrrole and probably myosmine. From this and estimation. of methoxy and methyl-imino groups, withanine was assumed to be monomethyl-imino pyridyl pyrrole derivative. The oxidation and hydrolysis gave no definite conclusions.

Kurup [28,29] isolated an antibacterial substance, "unsaturated lactone", $C_{24} H_{36} D_6$, m.p. 157-159°, from the leaves of *Withania somnifera* and described without any assignment of structural formula. Sastry et al[62] isolated withaniol, m. p.300° (earlier reported by Power and Salway[57]), hentriacontane and dulcitol, m. p. 185°.

Following these preliminary reports on the constituents of *Withania somnifera*, several manuscripts appeared concerning the chromatographic evaluation of extracts of the plant. Mario-Covello *et al*[9] reported the existance of eight alkaloids, in the roots of this plant, by paper chromatography. Dhalla *et al*[10] while conducting a comparative study of *Withania somnifera* and so called *Withania ashwagandha*, reported the presence of six and seven Dragendorff positive areas respectively. Later Rother *et al*[60] reported the presence of eleven nitrogeneous substances by paper chromatography in contrast to eight alkaloids reported earlier. In these reports, the authors, only confirmed the presence of choline and this fact was subsequently established by Khanna[21]. Khafagy *et al*[18] confirmed these findings and also reported the presence of two additional bases by thin layer chromatography. Atal *et al*[7] revealed the presence of sixteen Dragendorff positive components, with the aid of two dimensional thin layer chromatography, as compard to thirteen substances reported earlier.[60, 18]

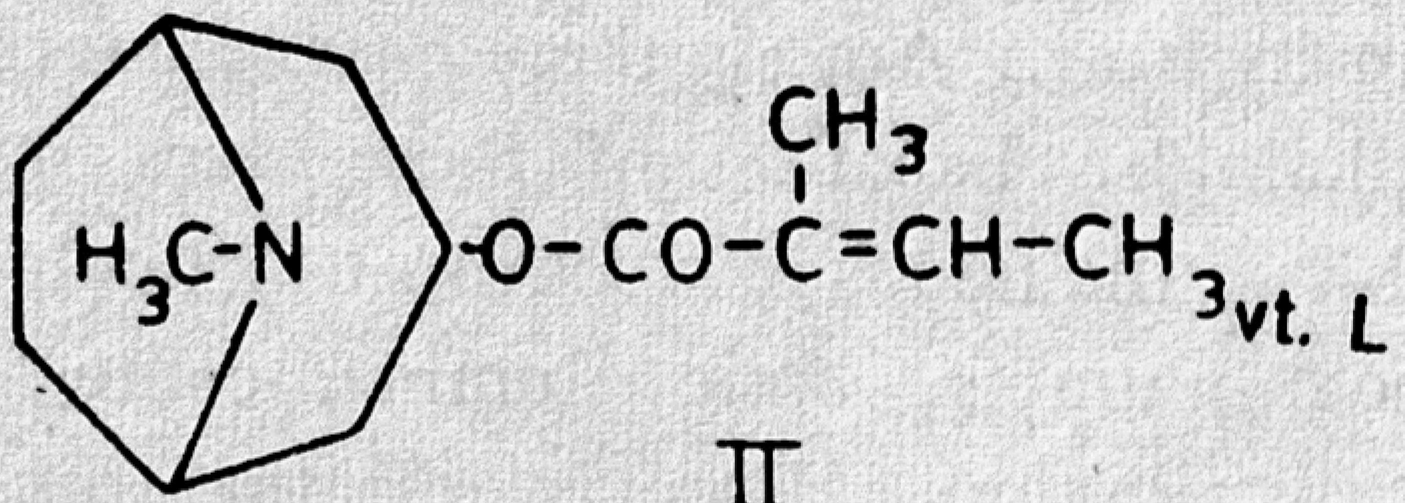
In the course of further investigation of the alkaloids of the roots of *W. somnifera*, a major break through was made by Schwarting *et al*[65] in the isolation and characterisation of eight bases present in the extract namely, tropine; pseudotropine; 3α-tigloyloxy tropane (3-tropyl tigloate); choline; cuscohygrine; dl-isopelletierine; anaferine and anahygrine. Among these bases anaferine and anahygrine are new alkaloids. Isolation of these bases involved all the methods available in the hands of a chemist like column chromatography over acid alumina, basic alumina, celite, cellulose and counter-current distribution. However, nicotine was not reported by these workers as was reported earlier. The authors have reported in detail the extraction-isolation methods together with the physical-chemical properties of these alkaloids and also reported the synthesis and some other properties of the two new alkaloids. A brief resume of the above mentioned alkaloids is as follows:

Tropine and Pseudotropine: (1) Khana *et al*[19] reported for the first time the presence of these bases in the genus *Withania*. From the alcoholic extract of the roots of *Withania somnifera*, these bases were obtained as a pair in the ion-exchange chromatography. The identity of the individual alkaloid was established through elemental analysis, derivatives, by comparative infra-red absorption spectra and by mixed melting points with the reference samples. Pseudotropine is stereoisomeric with tropine.



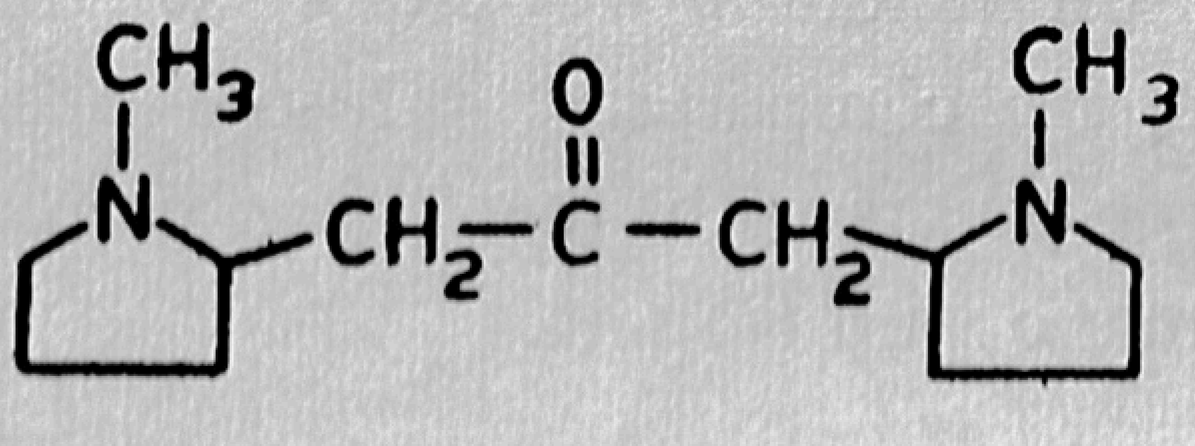
I

3-tropyl tigloate: (II) Leary et al[42] obtained it by column chromatography of the chloroform extract. m.p. 181.5-183°. It gave 3-tropyl tigloate hydrocloride, m.p. 214.5-217.5°; picrate, m.p.181.5°-183°. The product on reduction yielded tropine-dl-γ-methyl butyrate. The identity of the alkaloid was established by its comparison with the synthetic product.



II

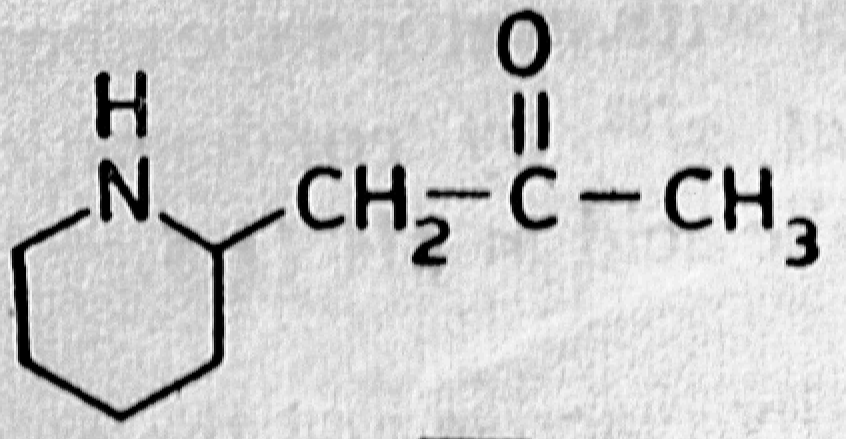
Cuscohygrine: (III) This alkaloid was also isolated by Leary *et al*[42] from the alcoholic extract of the roots by ion-exchange chromatography, followed by counter current distribution. It is a dark brown viscous oil. It gave dipicrate, m.p.224°; distyphnate, m.p.-203°. Its identity was established by comparative infra-red absorption spectra and mixed melting point data of the dipicrate and comparative Rf data of the dihydrochloride both singly and in mixture with authentic samples.



III

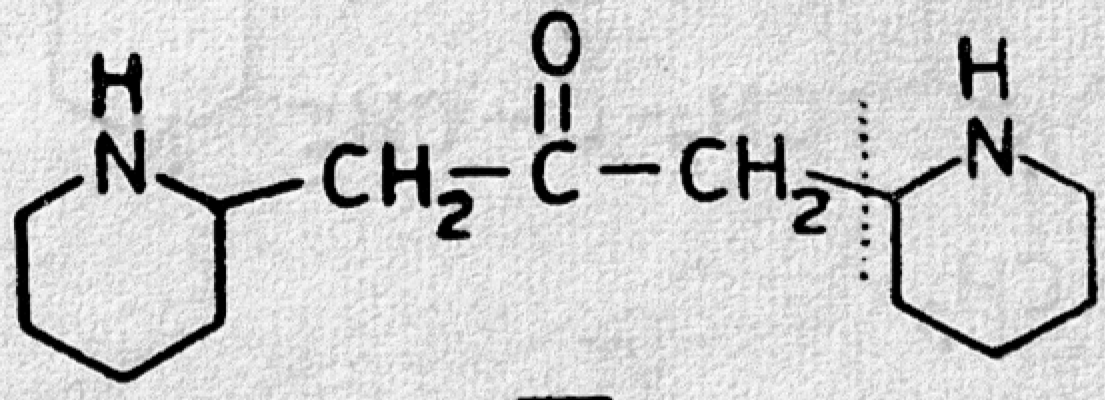
dl-Isopelietierine: (IV) It was isolated as isopelletierine hydrochloride, m.p.145°, by Khanna *et al*[20] from the alcoholic extract of the defatted, granulated roots of *Withania somnifera* by ion exchange chromatography followed by column chromatography over acid alumina. It gave picrate, m.p.148.5°-149.5°, and 2, 4-dinitrophenyl hydrozone hydrochloride, m.p.240-241.5°. The identity of the isopelletierine was established through comparative and analytical studies of the hydrochloride prepared from the regenerated base, the picrate and the dinitrophenyl hydrazone hydrochloride of the isolated product with the authentic samples of natural and synthetic origin.

The occurrance of this alkaloid in the family Solanaceae was reported for the first time by Mortimer *et al*[52] from the leaves of *Duboisia myoporoides* R.Br.



IV

Anaferine: (V) Anaferine was reported by Rother *et al*[60] for the first time as a naturally occurring compound. It was isolated as its dihydrochloride, $C_{13}H_{26}Cl_2N_2O$,m.p.222.5-223.5° from an alcoholic extract of the roots of *Withania somnifera* by ion-exchange chromatography and counter current distribution.



V

Anaferine, itself $C_{13}H_{24}N_2O$,b.p.55°/0. 01/mm,is a hygroscopic colourless oil which gradually discolours in air and gives a poor elemental analysis. It was characterized through three crystalline salts; a dipicrate, $C_{25}H_{30}O_{15}N_8$; m.p. 184-185°, a distyphnate, $C_{25}H_{30}N_8O_{17}$, m.p. 229-231°, and a dihydrochloride.

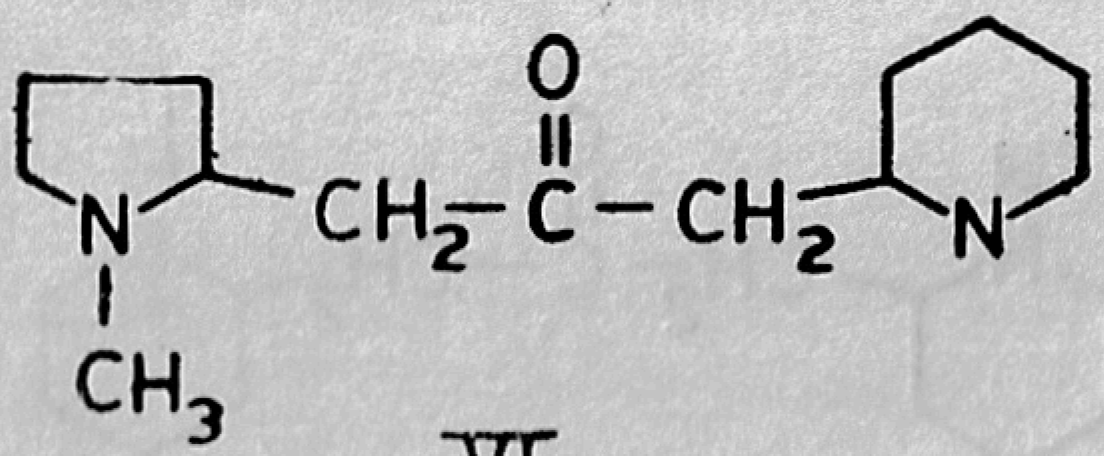
The I.R. spectrum anaferine has peaks at 3270 cm^{-1} (N-H), 1710 cm^{-1} (ketone C=O) and at 1640 cm^{-1} (amide carbonyl). It is a disecondary amine and forms a N-N-diacetyl derivative, $C_{17}H_{28}N_2O_3$, b.p. 120°/0.02 mm. The mass spectrum of anaferine gave the highest peak at 84 corresponding to the piperidine fragment formed by the cleavage of (V).

N. M. R. shows peaks at 7.083*T* (six protons adjacent to nitrogen), at 7.65*T* (doublet, protons α to carbonyl) and at 8.55*T* (12 protons on ring carbon not adjacent to nitrogen).

From these data and biogenetic considerations, the authors assigned anaferine, the structure of 1, 3-bis (2-piperidyl)-propane-2-one which was confirmed by synthesis. This structure was first reported by Anet *et al*[2] in the synthesis of sparateine by condnesation of 5-amino pentanal with acetone dicarboxylic acid at pH 11 and later by Schopf *et al*[63] by condensation of Δ' -piperideine with acetone dicarboxylic acid at pH 11.5.

Anaferine is optically inactive and the evidence obtained by the authors leads to conclude that it is probably meso.

Anahygrine: (VI) Anahygrine, $C_{13}H_{24}ON_2$, [1-(1-methyl-2-pyrrolidyl)-3-(2-piperidyl)-propan-2-one] another new alkaloid was reported by Leary *et al*[43] for the first time as a naturally occurring compound.



VI

Anahygrine, isolated from the alcoholic extract of *W. somnifera* is also a colourless oil which gradually darkens during storage. It gave hydrochloride, $C_{13}H_{26}Cl_2N_2O$, m. p. 216-217.5° and a dipicrate $C_{25}H_{30}N_8O_{15}$ m. p. 173-174.5°. The elemental analysis of the hydrochloride and of the picrate were used to establish its formula. These analytical data demonstrated its equivalency to both anaferine and cuscohygrine, although it possessed only 1 N-Me group where as the other two alkaloids had none and two respectively.

Evidence of the N-Methyl group located on the pyrrolidine ring was obtained by Mass spectrometry. The I. R. spectrum showed the presence of a carbonyl function at 1725 cm^{-1}. The structure of anahygrine was further cofirmed by synthesis by the condensation of ethyl-1-methyl-2.pyrrylacilate with 2-picolyllithium.

Paper chromatographic distribution of eleven nitrogenous substances of the total alkaloidal mixture of *Withania somnifera*, reported by Schwarting *et al*[65] is shown in Table III.

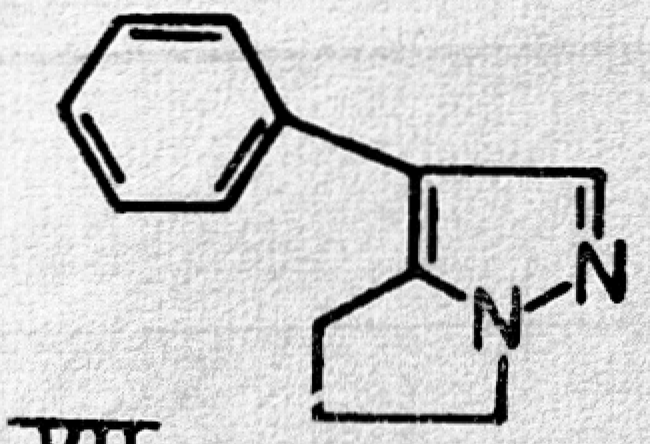
TABLE III

Alkaloids	Rf
1. Cuscohygrine	0.07
2. Choline	0.10
3. Anahygrine	0.14
4. Pseudotropine	0.20
5. Anaferine	0.23
6. Tropine	0.35
7. Isopelletierine	0.43
8. Alkaloid, not isolated	0.53
9. Alkaloid, not isolated.	0.69
10. 3-tropyl tigloate.	0.84
11. Dragendorff-positive steroid and unknown alkaloid	0.92

System: Whatman No. 1 paper, impregnated with 0.5 M KCl solution, dried and developed with n-butanol-HCl (98:2v/v), water saturated, descending.

Schroeter *et al*[64] reported the isolation of withasomnine, a pyrazole type of alkaloid from the roots of *Withania somnifera*, grown in Halle.

Withasomnine : (VII), $C_{12} H_{12} N_2$, is a crystalline base, m. p. 117-118° and was isolated from the ether extract of the roots by ion-exchange and column chromatography. It gave a picrate derivative, m. p. 170-173°; aurichloride, m. p. 203°. Treatment with HCl gave a very hygroscopic hydrochloride, m. p. 115-117°. Withasomnine gives a red colour with Munier's reagent. It does not react with hydrogen iodide and cannot be acetylated.

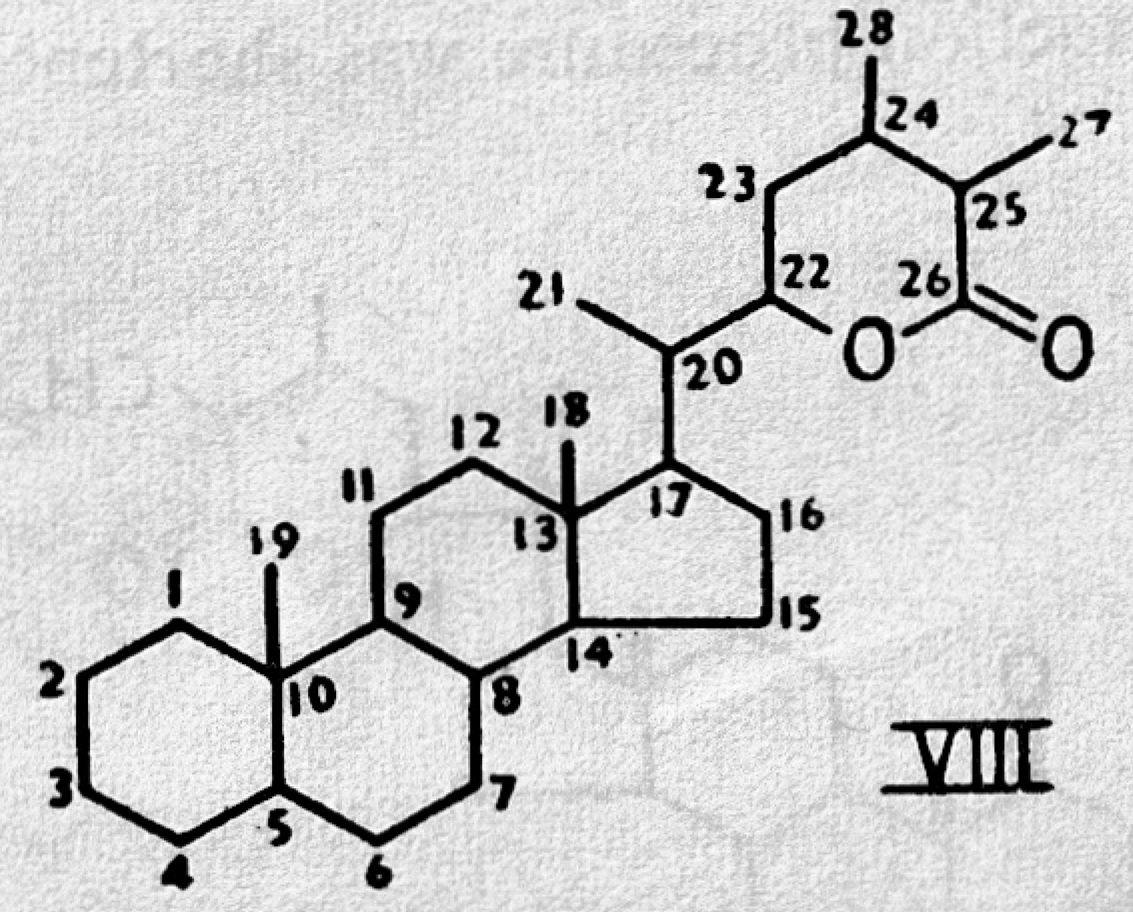


VII

In the i. r. spectrum, withasomnine has bands at 3000 cm^{-1} 2900cm^{-1}(aromatic and aliphatic CH respectively); 1610, 1505, 770, 690cm^{-1} (a mono substituted benzene ring); 1570 and 1420 cm^{-1} (conjugated C=N and CH_2 respecttvely). NMR and Mass spectra gave all the necessary data for the elucidation of its structure.

This alkaloid was not identical with any of withania alkaloids reported earlier by different workers. From its spectral analysis and derivatives, withasomnine was assigned the structure of 4-phenyl-1, 5-trimethylene pyrazole (VII).

Besides the alkaloids reported from the roots, some non alkaloidal constituents have been isolated from the leaves of this plant. A majority of these compounds belong to a new group of steroidal lactones for which the general name "withanolide" has been proposed by Lavie. *et al*[36], [38]. These compounds are characterized by a C_{28} basic skeleton with a 9 carbon atoms side chain having a six membered ring lactone (VIII). From the biogenetic point of view, the withanolides can be considered as possessing a highly oxygenated cholestane type side chain bearing an extra methyl group at C-24. More than 35 such compounds have been isolated so far from different Solanceae.



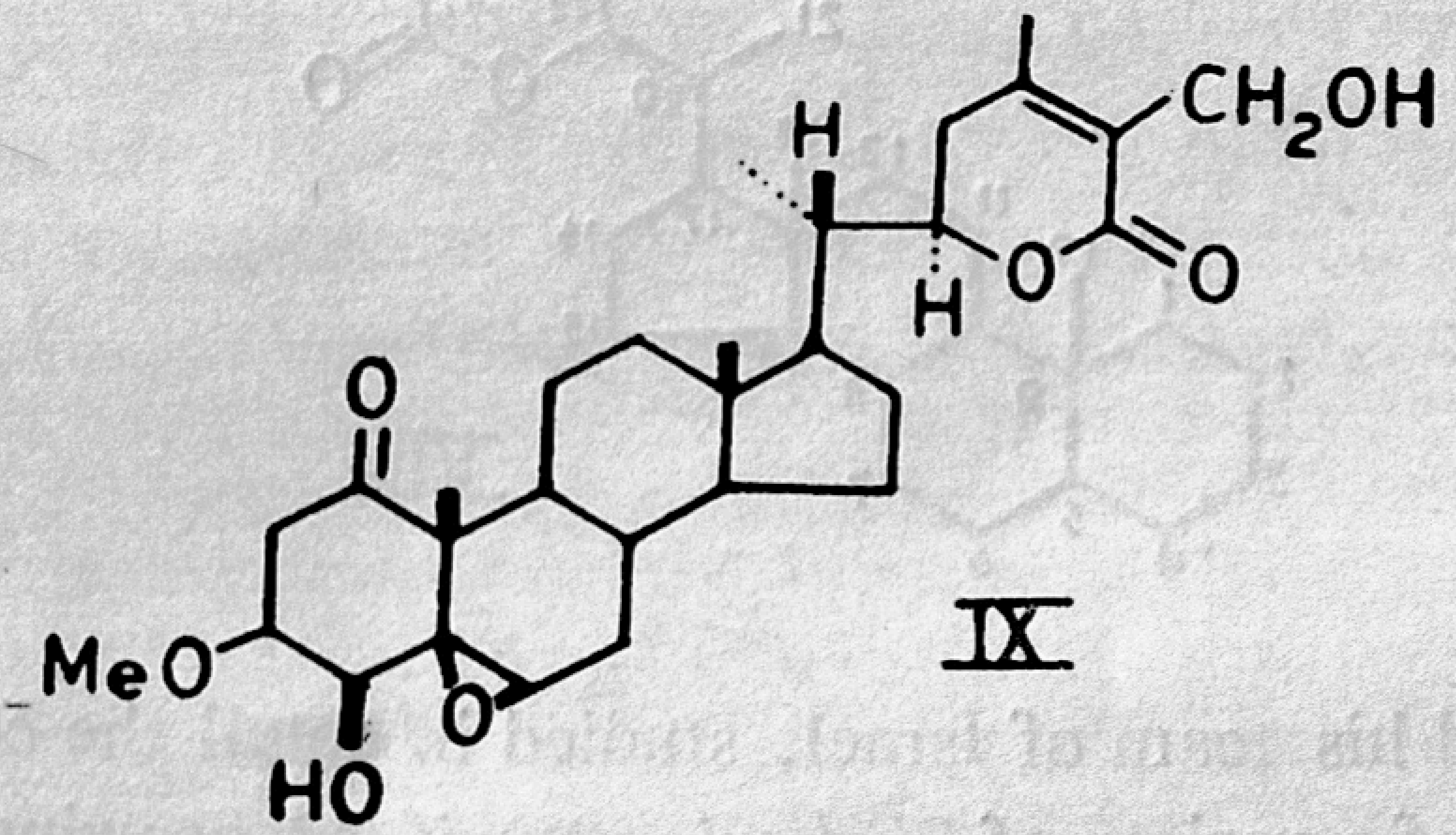
VIII

Lavie and his team of Israel, studied in detail the constituents of the leaves of a variety of *Withania somnifera*, growing in different localities of Israel, India and S. Africa. They isolated several new steroidal lactones, following a careful and systematic chromatography of the crude methanolic extract of the leaves of this plant, and published a series of papers on the constitution of *Withania somnifera*. Their first paper appeared in the year 1962[77] when they reported the isolation of five crystalline compounds, namely A_1, A_2, A_3, A_4, and A_5 and identified compound A_1 as withaferin. These compounds were also shown to possess antibacterial activity against aerobic bacilli[13].

The various withanolides isolated so far by different workers from the leaves of *Withania somnifera* are as follows.

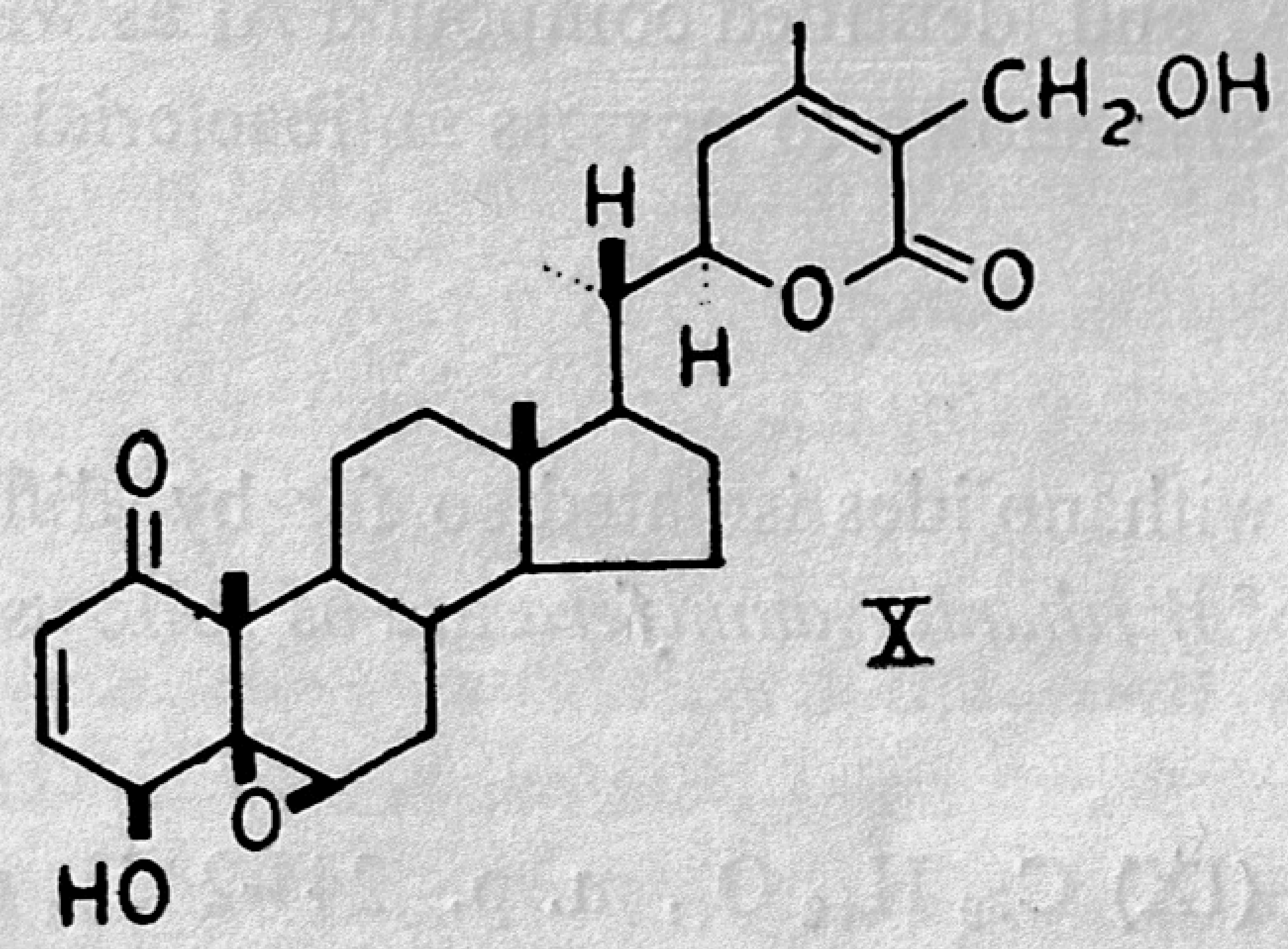
Withaferin : (IX) $C_{29}H_{42}O_7$, m. p. 244-245°, this compound previously described as A_1 was isolated by Asher yarden[77] and D. lavie[34] from the methanolic extract of the leaves of *W.somnifera*. It was identified as 2, 3-dihydro-3-methoxy withaferin A. The structure of this compound has been elucidated by analysis of the spectral data, as well as its different derivatives.

These authors reported that withaferin had turned out to be an artefact and was formed from withaferin A; another new steroidal lactone, during the prolonged extraction of the plant material with industrial methanol. This compound could not be obtained again, when the extraction procedure was shortened, or performed with ethanol.



IX

Withaferin A : (X) :—C_{28} H_{38} O_6. m. p. 243-245; [λ] D 114°, † max 214 nm (←17, 1500); V max 32-37 1692 cm^{-1} (carbonyl region). This compound previously designated as A_2 has been identified as 4β, 27-dihydroxy-1-oxd-5β, 6β-epoxy-22 R-witha-2, 24-dienolide.



X

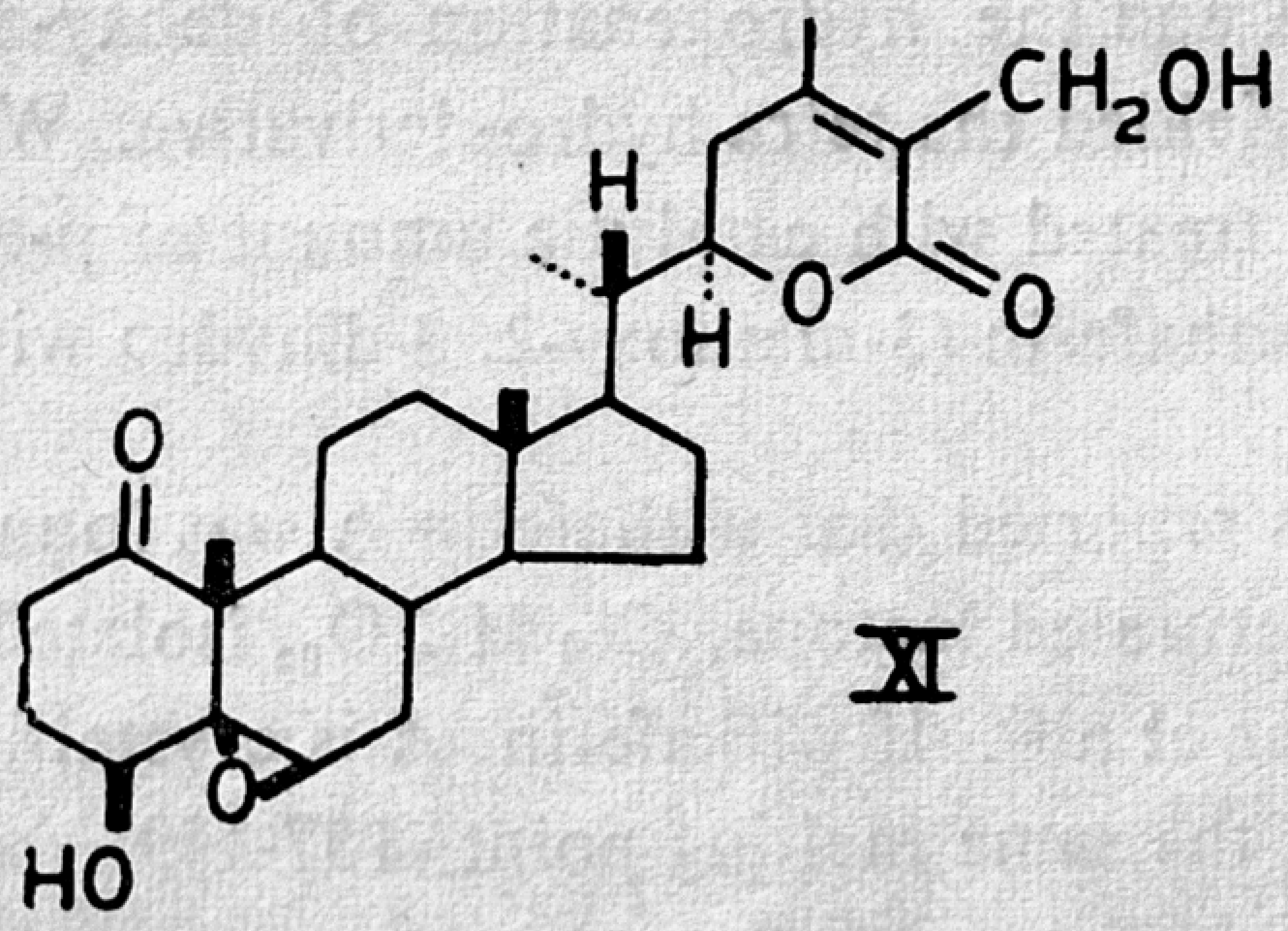
The structure of this compound was deduced by spectroscopic evidence as well as chemical degradation. Several selenium dehydrogenation expeiments yielded a cyclopenteno-phenanthrene and trimethyl naphthalein derivatives. Hydrogenation of withaferin A gave dihydro withaferin A. (This compound is identical with the naturally occurring compound designated as A_3). Hydrogenolysis of the primary hydroxyl group led to the formation of deoxydihydro withaferin A and the hydrogenation of deoxy-dihydro withaferin A acetate yielded the tetra hydro-derivative. Withaferin A (in methanol) when treated with catalytic amount of p-toluene-sulphonic acid yields withaferin (3-methoxy-2, 3-dihydro withaferin A).

The authors reported that withaferin A was found to be identical with the unsaturated lactone, $C_{24}H_{36}O_6$, isolated by Kurup[28,29] and Kholmuenzer et al[25]. If withaferin A is crystallized from ethanol-water, it has the same melting point 157-159°, as described by Kurup.

Withaferin A is 5β-steroid with the usual stereochemistry at all asymmetric centres, possessing a 6-membered ring lactone in the side chain. The authors have also synthesized several model steroidal 5, 6-epoxy derivatives bearing an oxygen substituent at C-4 in connection with their studies on the stereochemistry of withaferin A in which rings A and B of the steroidal molecule have a rather unusual substitution pattern. The β-orientation of the 5, 6 epoxide has been demonstrated by optical dispersion and NMR. The rearrangement of withaferin A, and some of its derivatives, has also been studied. Under acidic condition (sulphuric acid in acetic acid) withaferin A undergoes a novel type of rearrangement where an unsaturated keto aldehyde is formed which further on deformylation yields 2, 5-dien-1-one with a total loss of one ring-A carbon atom. The mass spectrum of six significant compounds of this series have been discussed by the authors.

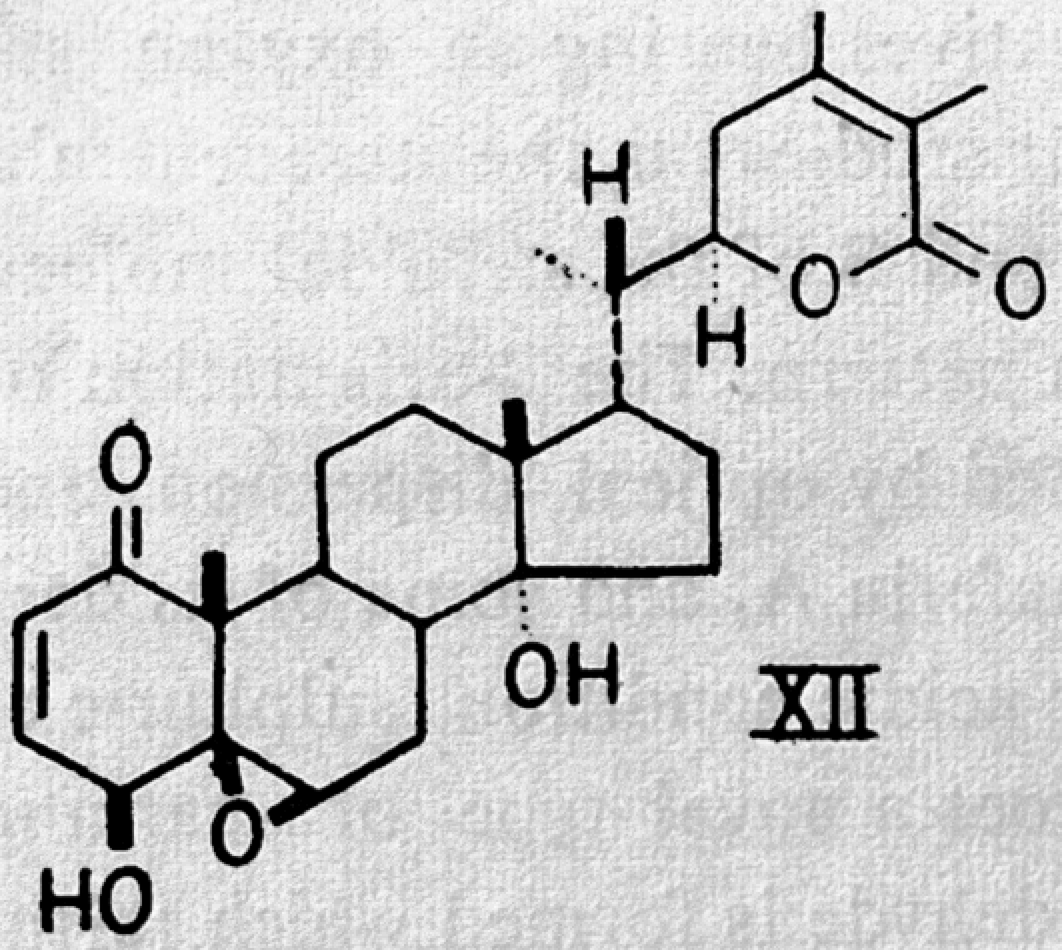
2 ,3-Dihydro withaferin A: (XI) :— $C_{28}H_{40}O_6$, m. p. 229-230°. This compound previously described as A_3, and isolated along with withaferin A was found to be dihydro derivative of withaferin A.

Kurup[28, 29, 31] first reported that withaferin A possesses a remarkable bacteriostatic activity and his findings were later confirmed by Efrain[13]. Kupchan et al[27] and Shohat[67], [68] described that withaferin A has also deen found to have antitumor properties.



XI

27-desoxy-14α -hydroxy withaferin A: (XII) :—This compound $C_{28}H_{38}O_6$, m. p. 265-267° was isolated by E. Glotter et al[14] from the methanolic extract of the leaves of *Withania* somnifera by coloumn chromatography. This is isomeric with withaferin **A**.



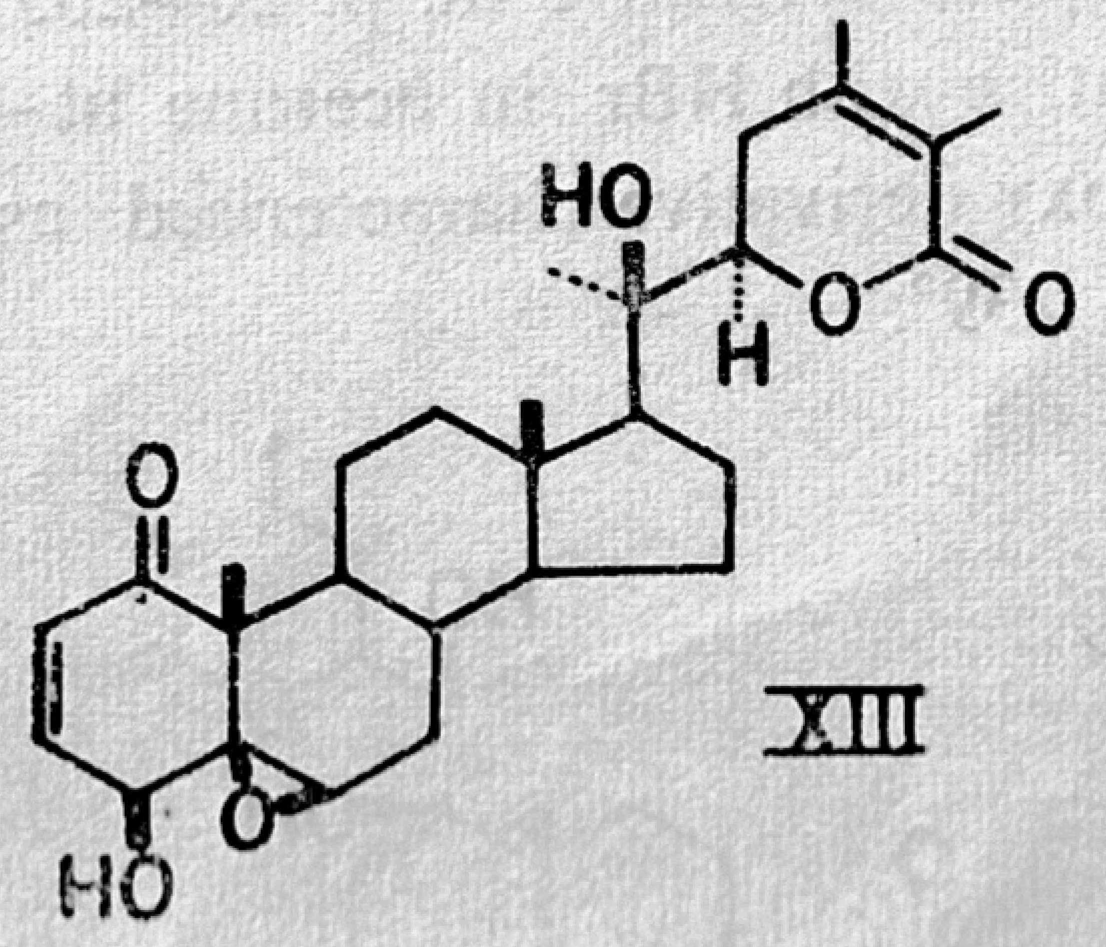
XII

The NMR spectrum of this new steroidal lactone reproduced most of the structural features of withaferin A and some of its derivatives. Identification has been confirmed by preparing the acetate, $C_{30}H_{40}O_7$, m. p. 268-269°, its dihydro product, m. p. 248-249°, various other derivatives and by the study of its spectral data.

Withanolide D : (XIII) **:—(27-desoxy-20** α **(R)— hydroxy withaferin A**).

It is another new steroidal lactone of withanolide type which has been isolated by D. Lavie *et al*[38], from the leaves of

Withania somnifera. This is a white crystalline substance, $C_{28}H_{38}O_6$, m.p. 253-255° (α) D+80°, λmax 217 nm ($\in$19,500); $V_{m}\alpha_{x}$ 1695cm^{-1} (carbonyl region). It is isomeric with withaferin A (X) as well with 27-desoxy-14-α-hydroxy withaferin A (XII).



XIII

Withanolide D has been identified as 5, 6β -epoxy-4-β-20-α-dihydroxy-1-oxd (5β) witha-2, 24-dienolide. The structure of this compound has been elucidated by its spectral analysis and by chemical degradation to 4β -acetoxy-5, 6β-epoxy (5β) pregan-1, 20-dione.

Glotter *et al*[15,40] reported the isolation of nine new steroidal lactones of the withanolide series (withanolide E-M) from the leaves of *withania somnifera*, chemotype III*[1,41] Compounds (IX-XII) and (XIII) described above have been isolated from chemotype I and II respectively.

An X-ray analysis studies have revealed that out of the new withanolides, two (E and F) have been found to possess the unsual 17α -oriented side chain. A brief resume of the nine steroidal lactones is as follows:

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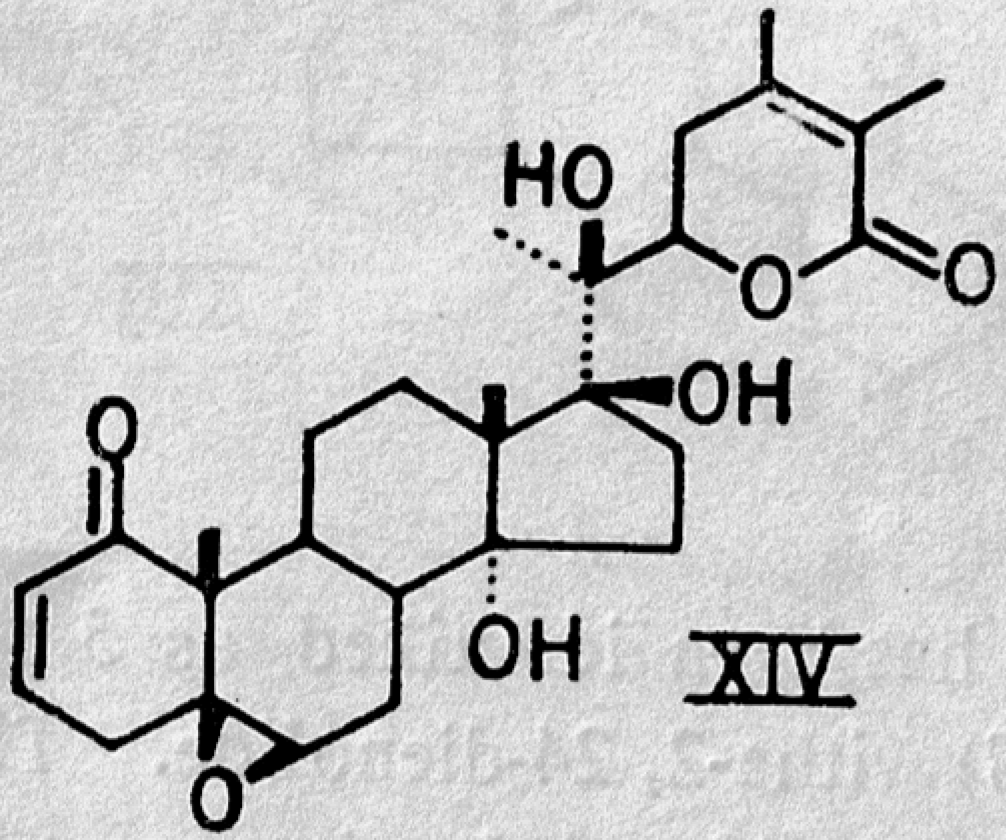
* [Abraham et al[1, 41] examined the leaves from samples of 24 populations of *Withania somnifera* growing in various parts of Israel, for their content in steroidal lactones. They described three chemical types of this species, each having different steroidal lactones of the "withanolide" type. They found the only variation of a chemical nature which was independent of the morphological characters of this species].

Withanalide E : (XIV) :—14α-17 β, 20 α-trihydroxy-5β, 6β-epoxy-1-oxo-17S, 20S, 22R-witha-2, 24-dienolide.

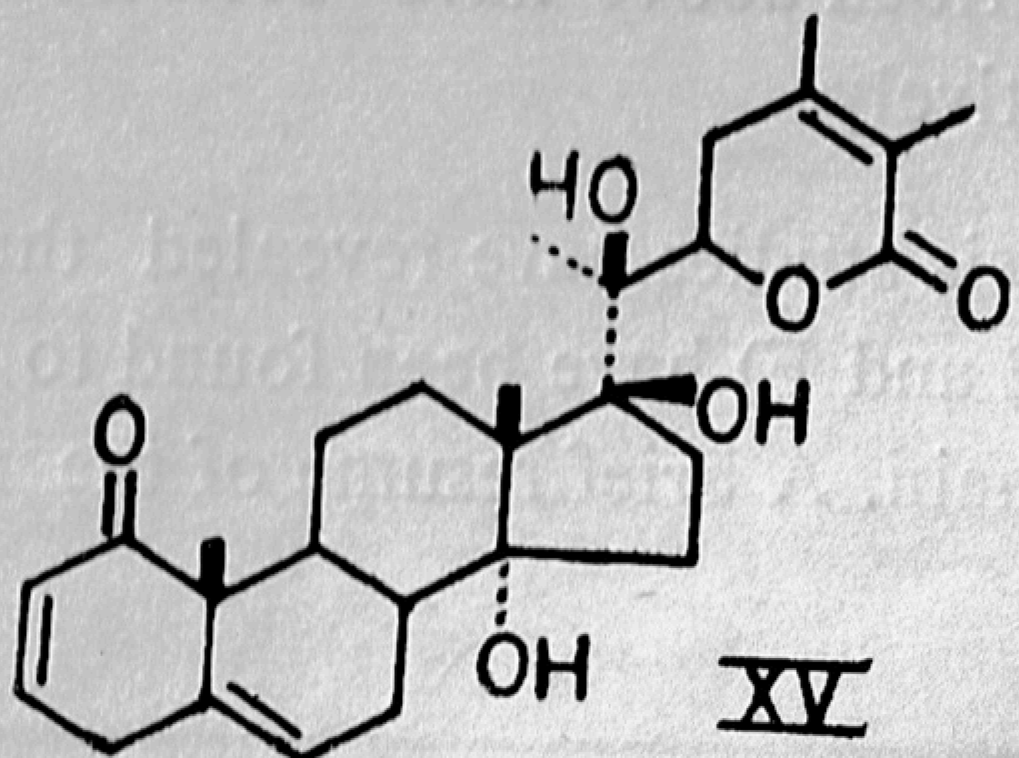
$C_{28}H_{38}O_7 . 2H_2O$, m. p. 167-168°, ν_{max} 1675 cm^{-1}, λ_{max} 223nm (Σ 17,000). Catalytic hydrogenation of the compound afforded a 2, 3-dihydro derivative m. p. 264-265°. Treatment of the 2-3-dihydro derivative with HBr in acetone at—12° gave the 5α-bromo - 6β -hydroxy derivative characterised as the correspoding mono acete, m. p. 239°.



XIV

Withanolide F : (XV) :—14 α, 17β, 20 α-trihydroxy-1-oxo-17S, 20S, 22R witha-2, 5, 24-trienolidc.

The tentative structures of withanolides E and F are mainly based on X-ray analysis. Details about final confirmation of their structure are still awaited.

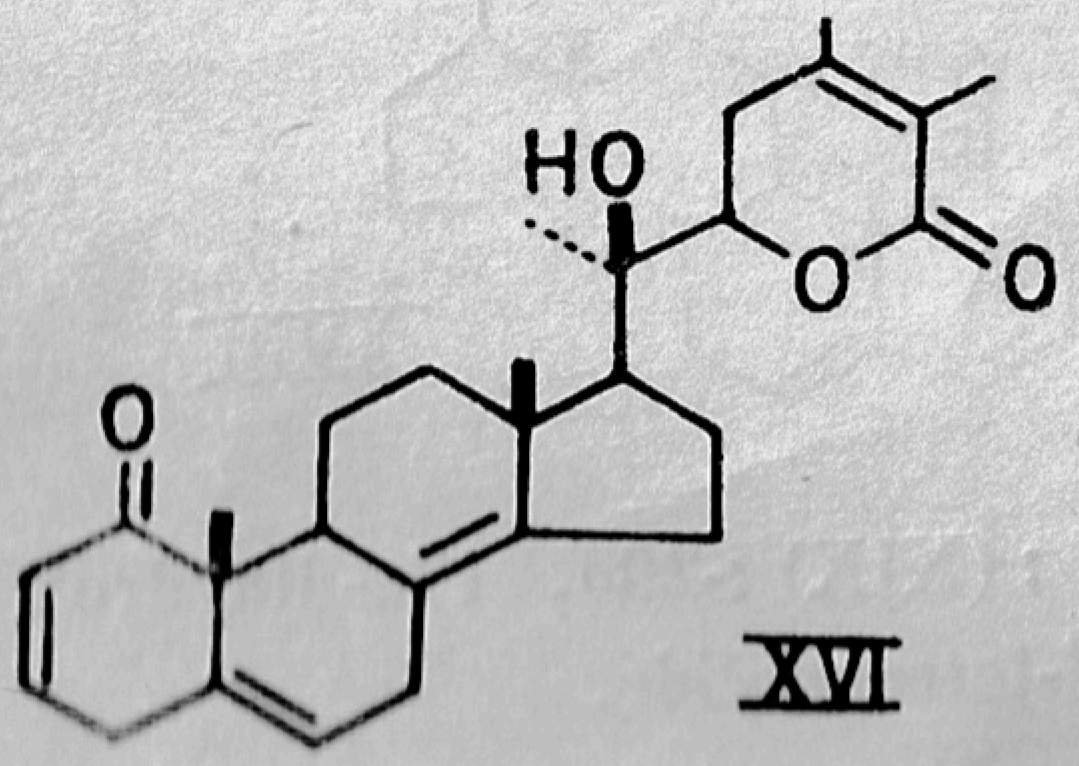


XV

Withanolide G : (XVI) :—20 α-hydroxy-1-oxo-20R, 22R-witha-2, 5, 8 (14), 24-tetraenolide.

It is a crystalline compound, $C_{28}H_{36}O_4$, m. p. 194-195°, [α]D+52.5°, ν_{max} 1685 and 1692 and cm^{-1} ; λ_{max} 223 nm

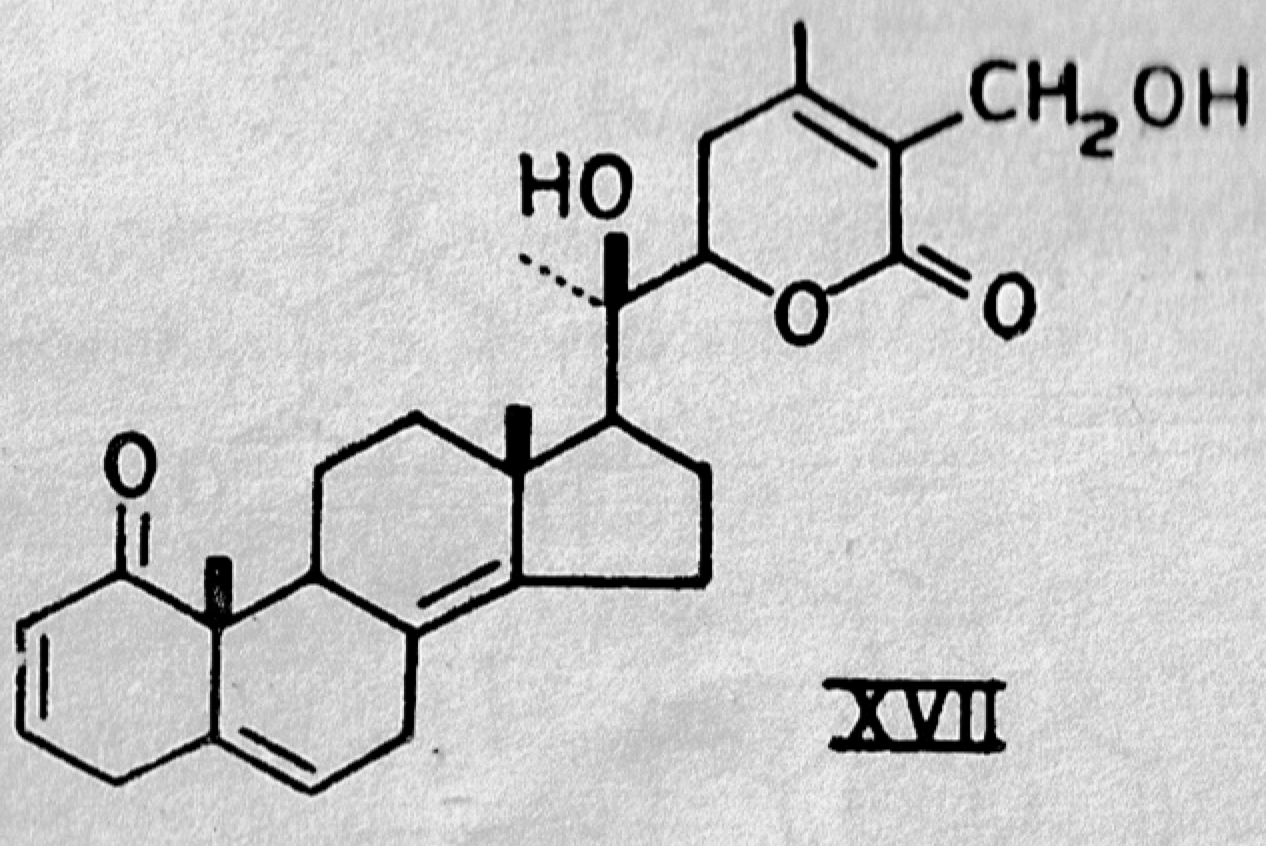
(Σ 19,400). Catalytic hydrogenation of the compound gave a 2,3-dihydro derivative $C_{28} H_{38} O_4$ m. p. 202°. Upon acetylation with acetic anhydride in pyridine, the compound remains unchanged, however, on treating with trichloroacetyl isocyanate, it afforded a mono carbamate.



XVI

Withanolide H : (XVII) :-20 α, 27-dihydroxy-1-oxo-20 R. 22R-witha-2,5,8,(14), 24-tetraenolide.

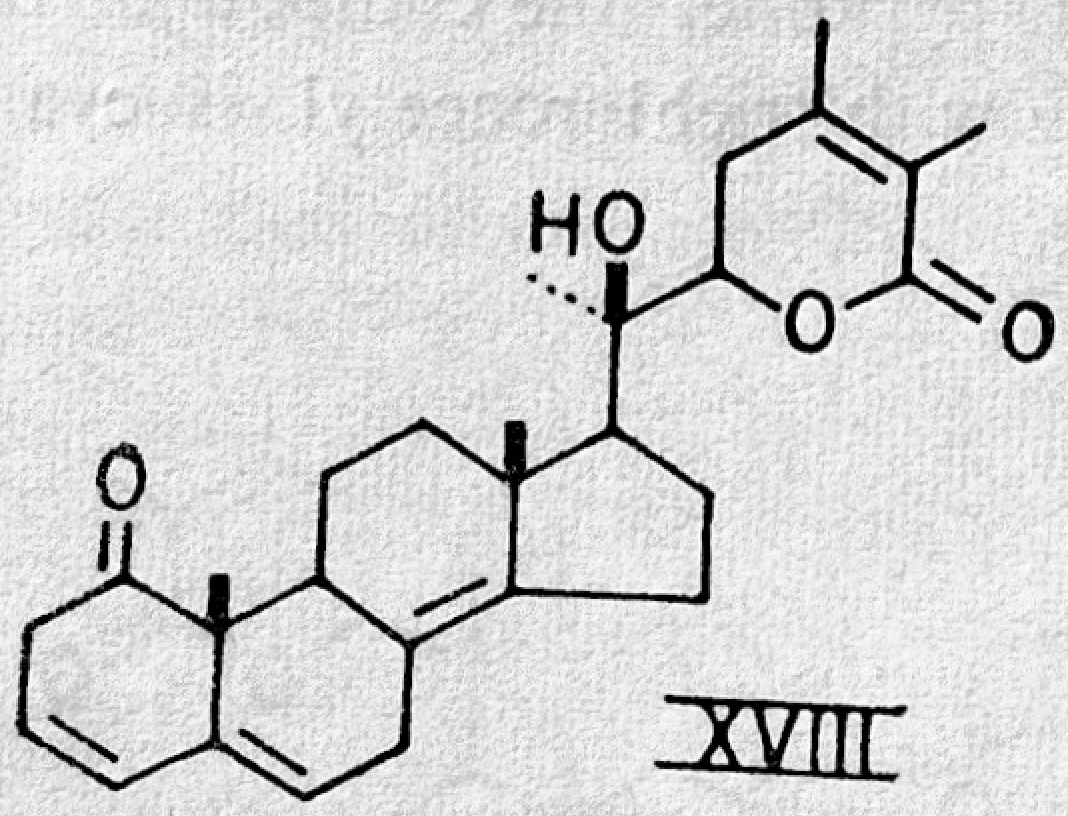
The compound $C_{28} H_{36} O_5$ (non crystalline) was characterized as its monoacetate, $C_{30} H_{38} O_6$, m. p. 141-142°, $[\alpha]D+35.5°$; $\nu_{m\alpha_x}$ 1689 and 1730 cm^{-1}; $\lambda_{m\alpha_x}$2 20 nm (∈17, 200).



XVII

Withanolide I : (XVIII) :-20 α-hydroxy-1-oxo-20R, 22R-witha-3,5,8(14), 24-tetraenolide.

$C_{28} H_{36} O_4$, m. p. 184°, $[\alpha]D+118°$ $\nu_{m\alpha_x}$ 1706 cm^{-1}. The compound possesses an intense uv band at 232 nm (∈24, 000), due to the overlap of the unsaturated lactone chromophore with that of the $\triangle_{3,5}$ heteroannular diene. This compound is isomeric with withanolide G(XVI), differing only in the position of the ring A double bond.

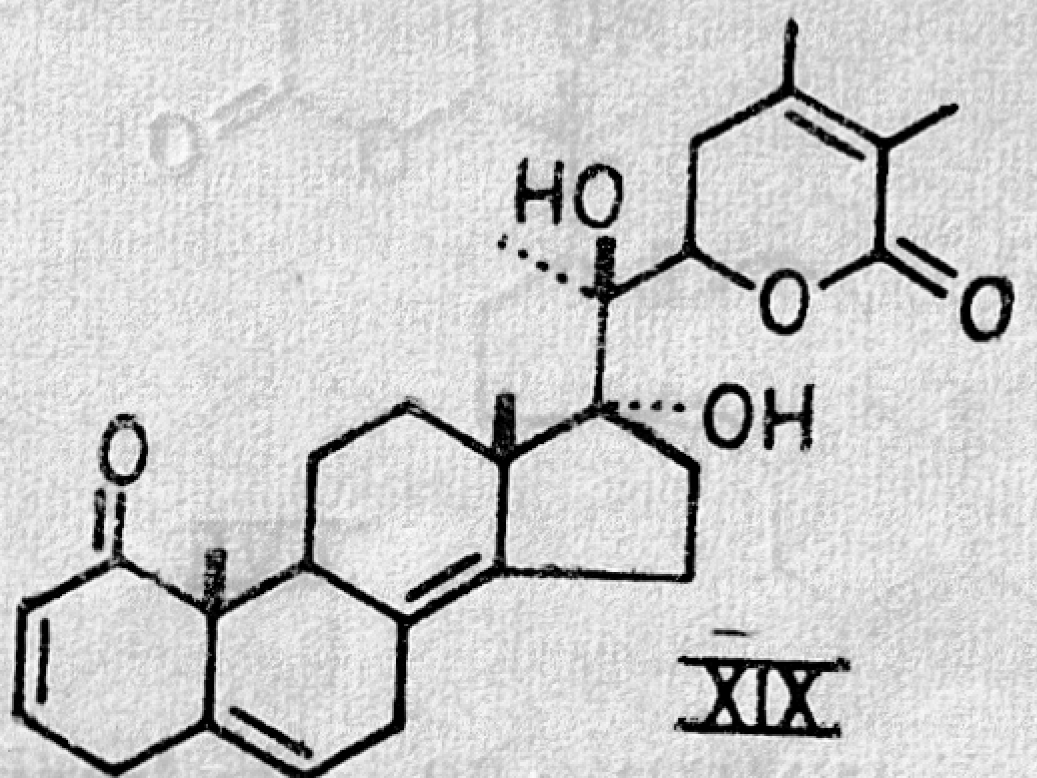


XVIII

Withanolide J : (XIX) :-20α, 17α-dihydroxy-1-oxo-20 S, 22R-witha-2,5,8(14), 24-tetraenolide.

$C_{23}H_{36}O_5$, m. p. 215-216°; $[\alpha]D+32.7°$; $\nu_{m\alpha_x}$ 1685 and 1692cm^{-1} ; $\lambda_{m\alpha_x}$ 224 nm ($\in$18,000).

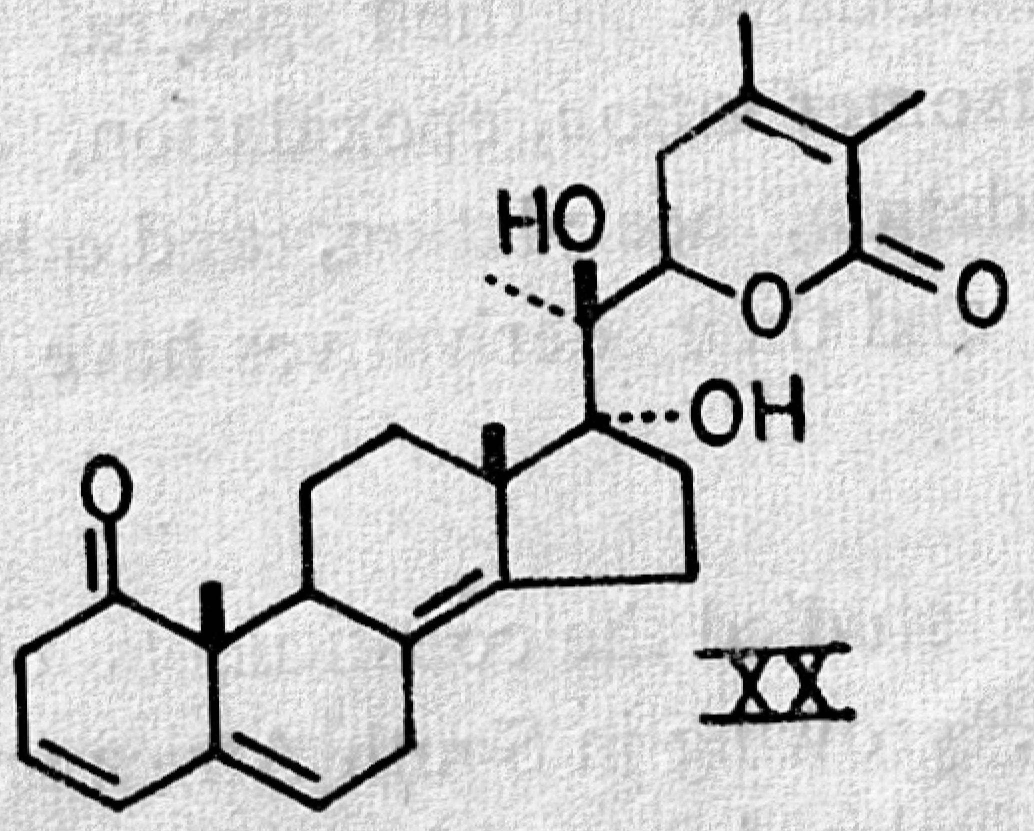
This compound possesses one tertiary OH group more than (XVI). Esterification of the compound with trichloroacetyl iscoyanate disclosed the presence of two OH groups due tot he appearence of a two proton signal at 88.96 for the trichloroacetyl carbamate protons.



XIX

Withanolide K : (XX) :—20 α, 17α-dihydroxy-1-oxo-20S, 22R-witha-3,5,8(14)-tetraenolide.

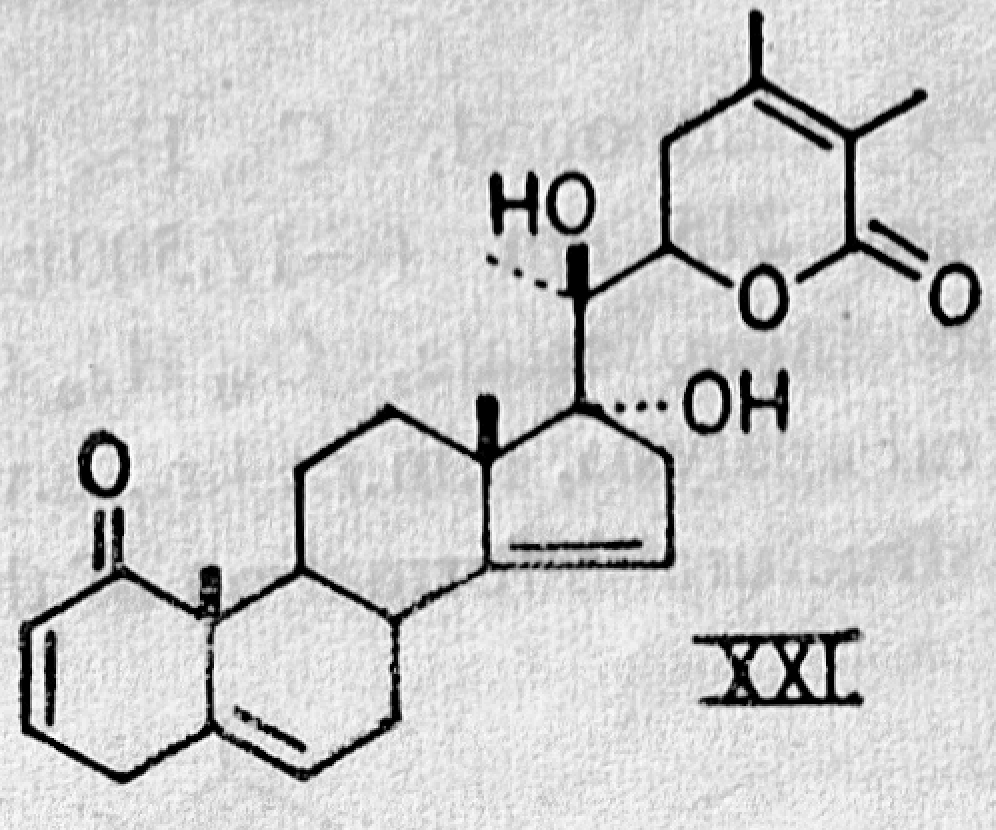
$C_{28}H_{36}O_5$ m. p. 218-219°, $[\alpha]D+92°$; $\nu_{m\alpha_x}$ 1706 cm^{-1}; $\lambda_{m\alpha_x}$ 231 nm ($\in$25,000). This compound is the 17α-OH analog of (XVIII) and its structure is supported by the similarity of their spectral data.



XX

Withanolide L : (XXI) :-22 α, 17α—dihydroxy-1-oxo-20S, 22R-witha-2,5,14, 24-tetraenolide.

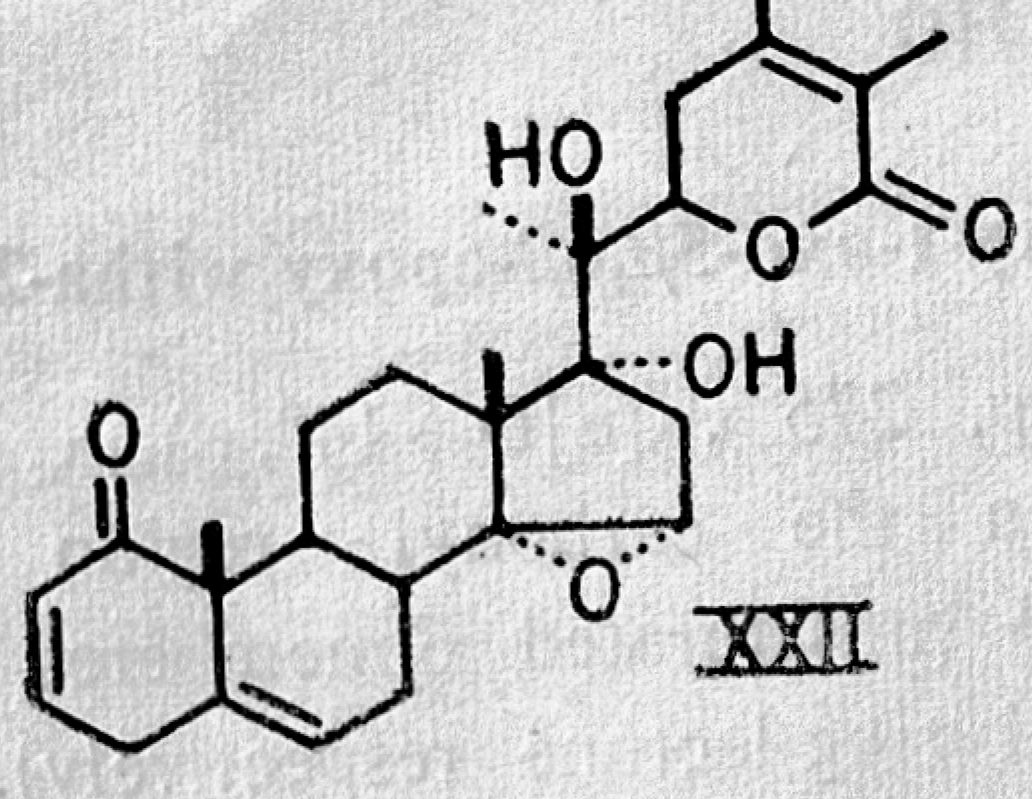
$C_{28}H_{36}O_5$, m.p. 213°, $[\alpha]D+9.6°$; ν_{max} 1698 cm^{-1}; λ_{max} 220 nm(sh) (∈18,600).



XXI

Withanolide M : (XXII) :—20 α, 17α-dihdroxy-14α, 15α-epoxy-20S, 22R-witha-2,5, 24-trienolide.

$C_{28}H_{36}O_6$, m. p. 240°, $[\alpha]D+44.6°$; ν_{max} 1692 cm^{-1} ; λ_{max} 225 nm (∈19,200). Epoxidation of the compound with per benzoic acid gave a maxture of 5,6 epoxides.



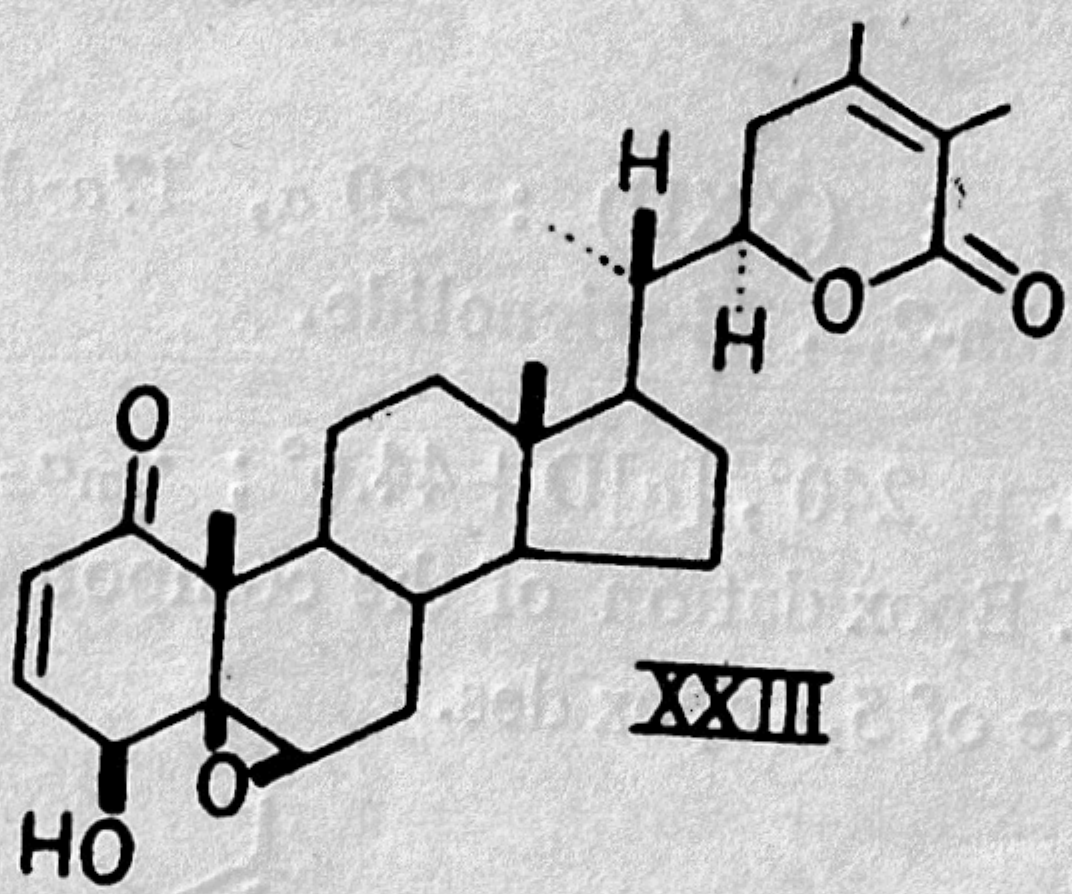
XXII

The sturctures of all the above mentioned seven wtihanolides (G-M) have been elucidated by their spectral analysis, e.g. n.m.r ; i.r.; v.u.; and m.s. Isomerisation, epoxidation, catalytic hydrogenatiou, reduction, oxidation, acetylation, and other chemical studies of the compounds and their derivatives have also been made by the authors.

Kirson et al[23],[39] studied the constituents of a different type of *Withania somnifera*, growing in South Africa and called it a South African type. They isolated five withanolides from the methonolic extract of the leaves of this plant; two of which were identified as withaferin A and withanolide D (compound X and XIII) and charetcrized the other three new withanolides as:

4-βhydroxy-1-oxo-5β,6β-epoxy witha-2,24-dienolide: (XXIII):—

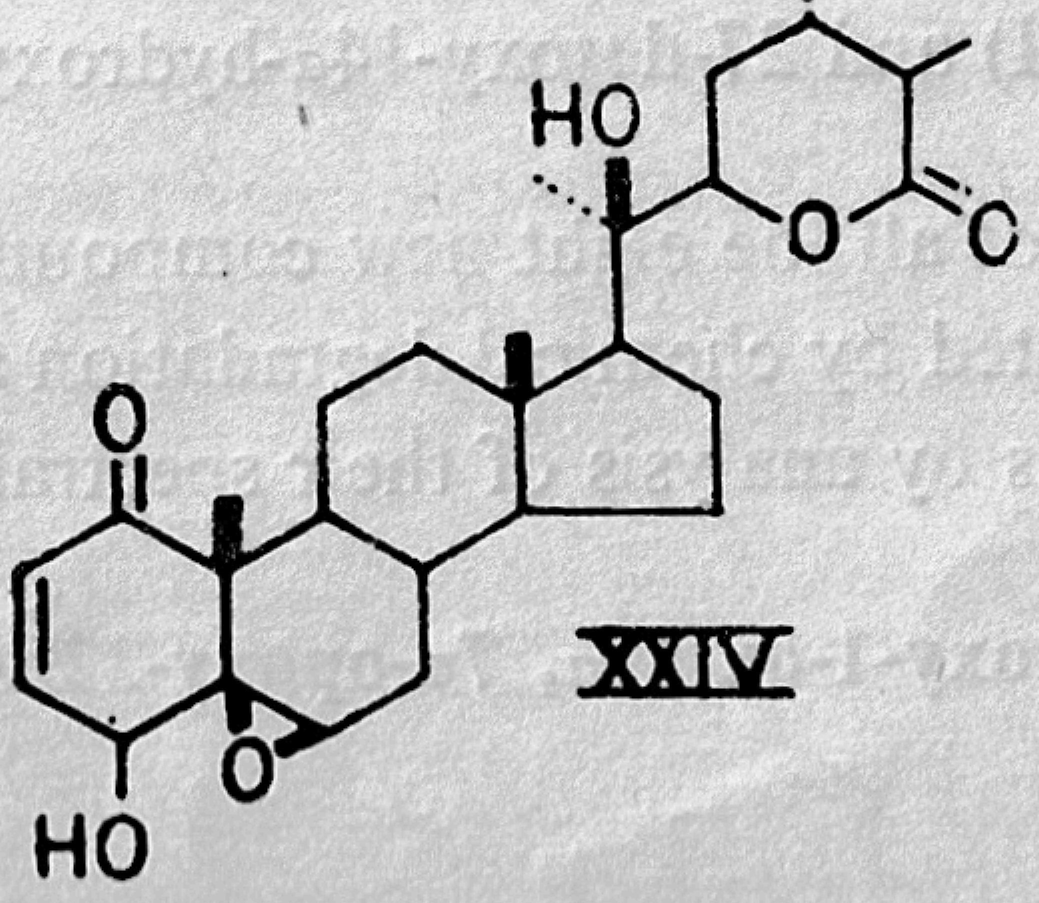
It is a crystalline compound, $C_{28}H_{38}O_5$, m. p. 268-269°, [α] D+101.5° ; λ_{max} 215 nm (←17,500); ν_{max} 1690 cm^{-1}. Upon acetylation, the monoacetate, $C_{30}H_{40}O_6$, m.p. 227-228°, was obtained. Spectral analysis, n.m.r., i.r., u.v. mass and derivatives afforded all the structural information of the characterization.



XXIII

4-β-20α (R) dihydroxy-1-oxo-5β,6β-epoxy witha-2-enolide : (XXIV):—

$C_{28}H_{40}O_6$, m.p. 275° , [α]D+14°, λ_{max} 214nm. The i.r. spectrum showed in the carbonyl region two bands at 1681 and 1730 cm^{-1} (unsaturated 6-membered ring ketone and saturated 6-membered ring lactone respectively). It gave a mono acetate derivative, $C_{30}H_{42}O_7$, m.p. 237-238°. The catalytic hydrogenation yielded the dihydroderivative.

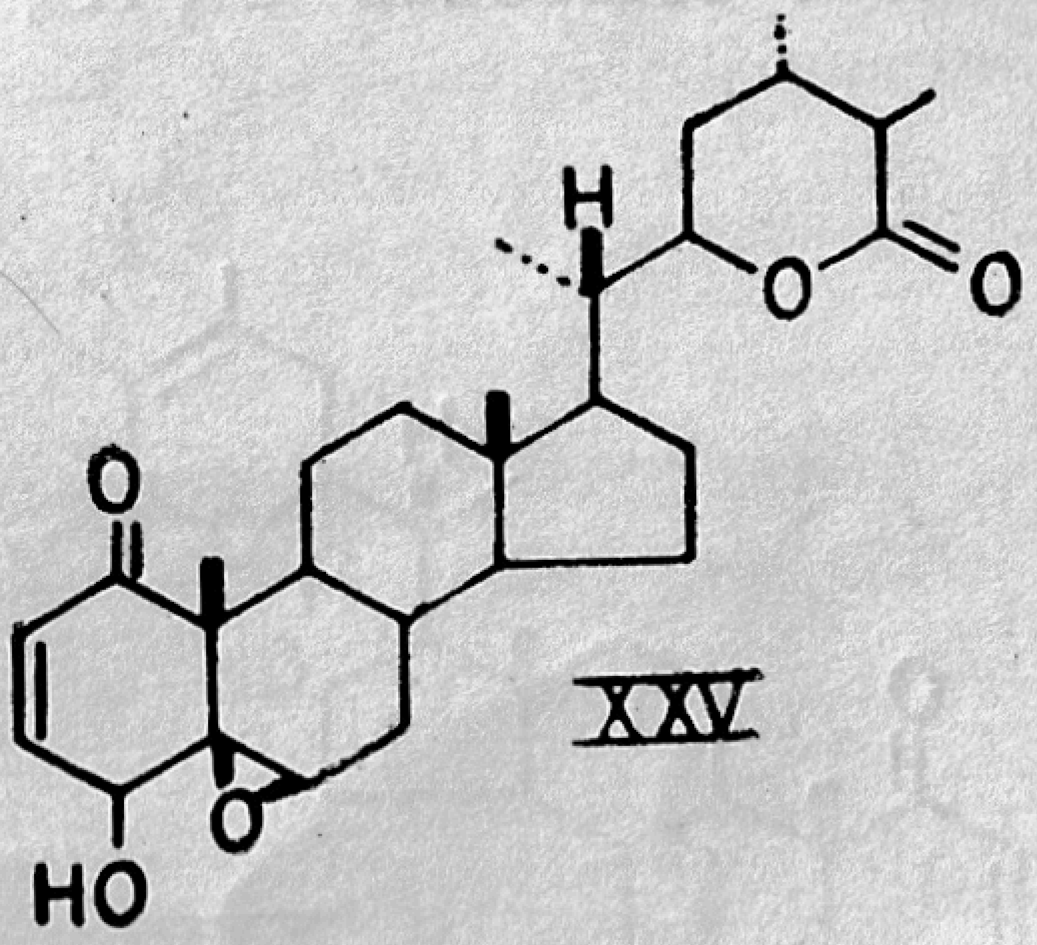


XXIV

The characterization of the compound was based on the spectral data as well as by degradation to the pregnane derivative.

§ and 4β-hydroxy-1-oxo-5β, 6β-epoxy witha-2-enolide : (XXV) :—

$C_{28}H_{40}O_5$ m.p. 252°, $[\alpha]D+32.5°$; λ_{max} 214 nm (∈9550), i.r. bands at 1730 and 1681 cm^{-1}. Upon acetylation it afforded the mono acetate derivative, $C_{30}H_{42}O_6$, m.p. 213°.



XXV

The structure of this compound was elucidated by the study of its chemical degradation to knownmocpounds an d supported by the spectral analysis (u.v;i.r.,n.m.r and m.s).

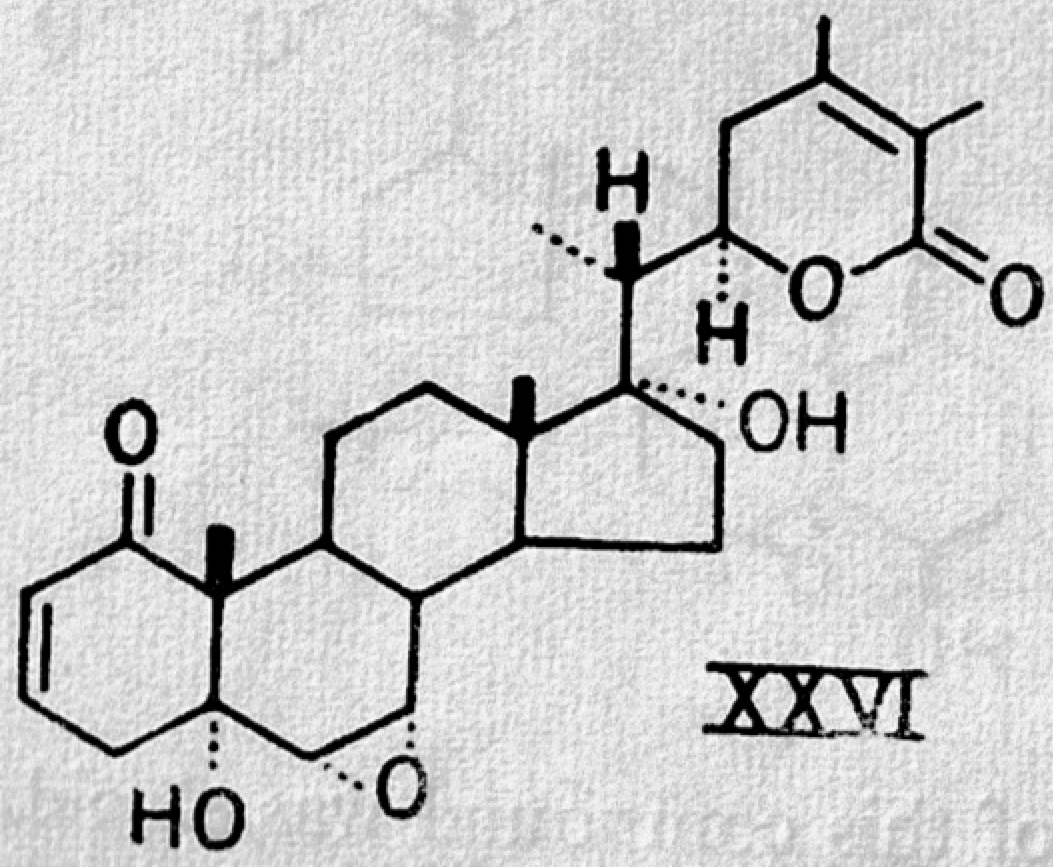
In a further communication, Kirson *et al*[24],[22] reported the isolation of eight new steriodal lactones from the leaves of

Withania somnifera, growing in North-Western India and called it chemotype 'India I'. Besides this they also identified 3 withanolides by direct comparison with already known withaferin A(X), 27-deoxy withaferin A(XXIII) and 27-desoxy-14α-hydroxy withaferin A(XII).

The structure of all the eight new compounds (XXVI-XXXIII) have been investigated by chemical degradation and interconversion reactions, as well as by analysis of their spectral properties.

5α, 17α-dihydroxy-1-oxo-6α, 7α-opoxy-22R-witha-2, 24-dienolide : (XXVI) :—

$C_{28}H_{38}O_6$, m.p. 275-276°, $[\alpha]D+81°$; $\lambda_{m\alpha_x}$ 223 nm (∈17,000); $\nu_{m\alpha_x}$ 1695 cm^{-1}. Catalytic hydrogenation of the compound gave a dihydroderivative $C_{28}H_{40}O_6$, m.p. 286-287°. Treatment of the dihydroderivative with hydrobromic acid yielded a mixture of two bromohydriny, $C_{28}H_{41}BrO_6$m. p. 204° and $C_{28}H_{41}BrO_6$, m.p. 256-258° respectively. Treatment of the compound with HI gave a iodohydrin $C_{28}H_{39}IO_6$, m,p. 198°. with excess of HI a reductive cleavage of epoxides takes place resulting into a trienone compound $C_{28}H_{36}O_4$, m.p. 249-252° (yellow needles).

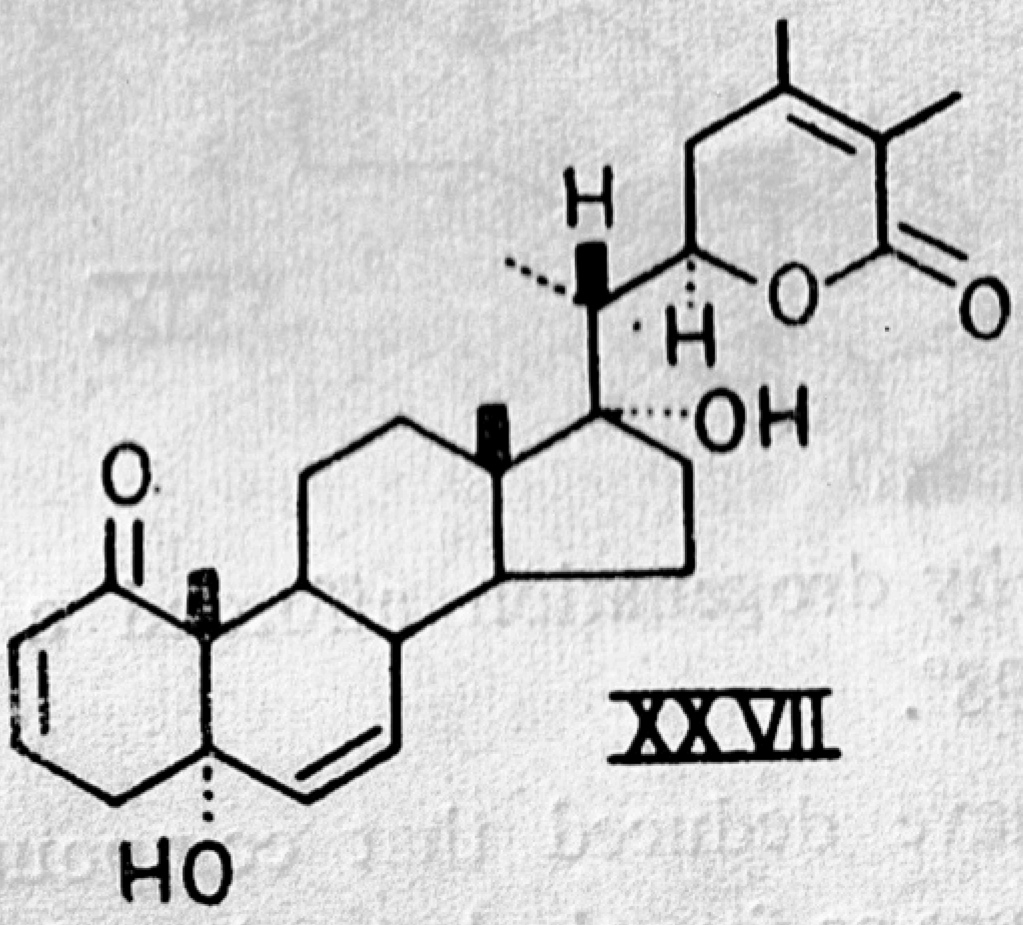


XXVI

The steroidal lactone of unknown structure, withanone, m.p. 263°, first isolated by Dhalla *et al*[11,70] from the leaves of *Withania somnifera* (collected in India, Delhi area) has been found to be identical with this compound.

5α,17α-dihydroxy-1-oxo-22R,witha-2,6,24-trienolide : (XXVII) :–

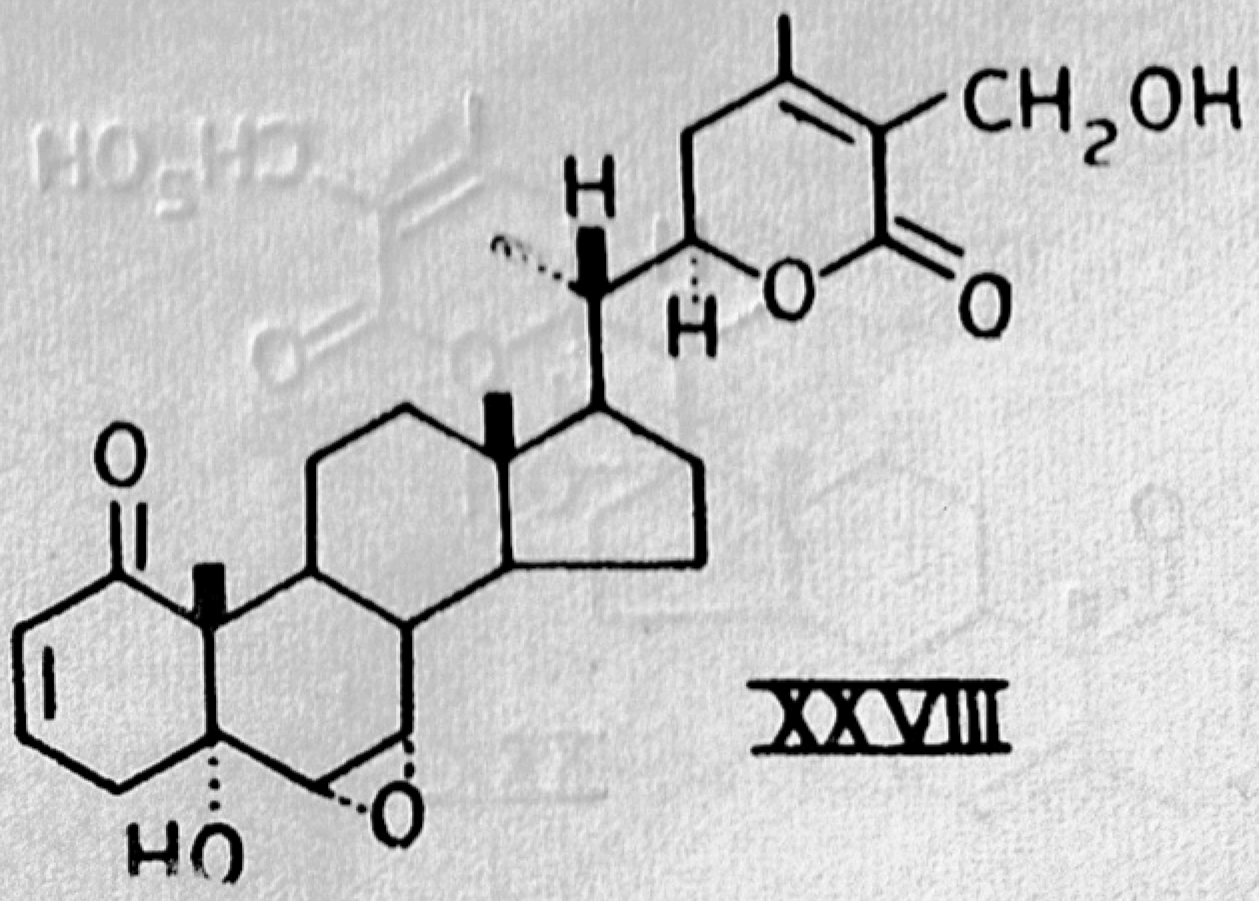
It is a needle shaped crystalline compound, $C_{28}H_{38}O_5$, m.p. 238-239°, [α]D+75.5°, λ_{max} 223 nm (∈16,500) ; ν_{max} 1695 cm^{-1}. On treatment with meta-chloroperbenzoic acid, it gave an epoxy derivative which was identified as compound (XXVI) by direct comparison. Dehydration of the compound with thionyl chloride as well as with sulphuric acid yielded a compound $C_{28}H_{34}O_3$, m.p. 201°.



XXVII

5α,27-dihydroxy-1-oxo-6α,7α-epoxy-22R-witha-2,24-dienolide : (XXVIII) :—

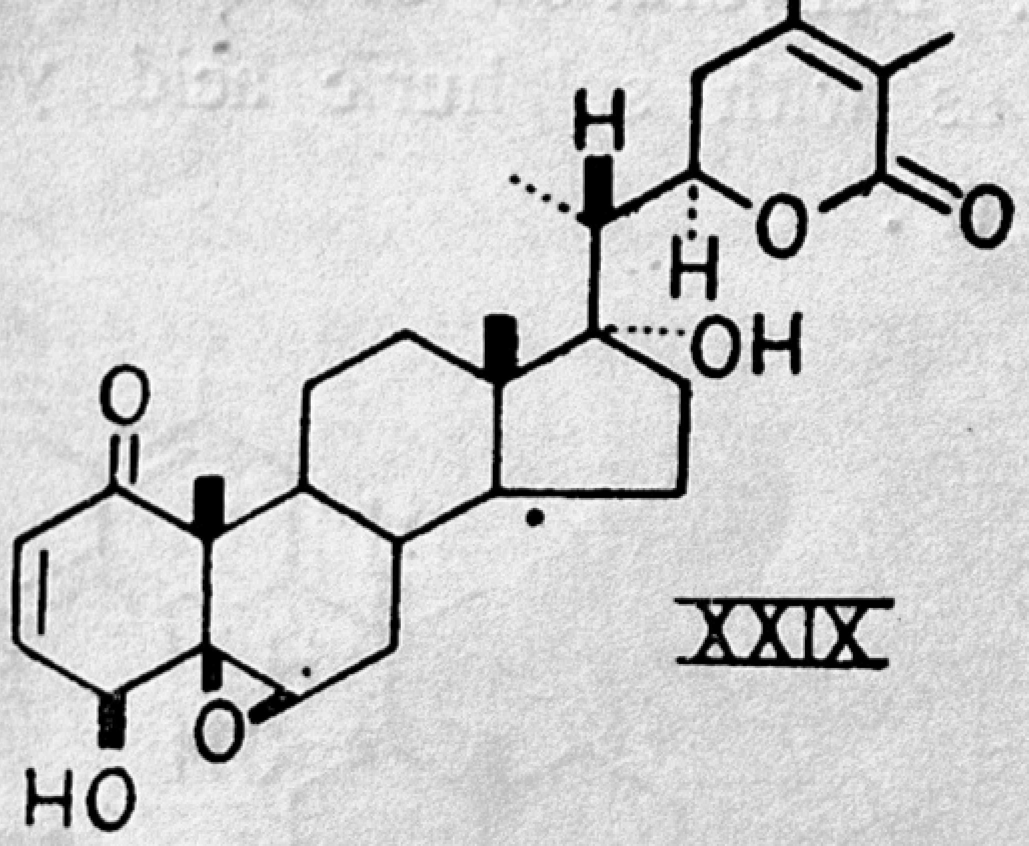
This compound has been isolated and characterized as acetate (obtained after acetylation of certain fractions from the eluates of the chromatography), $C_{40}H_{30}O_7$, m.p. 230°; [α]D+73°; λ_{max} 221.5nm; ν_{max} 1739, 1695 and 1227 cm^{-1}. Catalytic hydrogenation of the compound gave a dihydrodeacetoxy derivative, $C_{28}H_{40}O_5$, m.p. 250-252°.



XXVIII

4β, 17α-dihydroxy-1-oxo-5β,6β-epoxy-22R—witha-2,24-dienolide : (XXIX) :—

This compound was also isolated and characterized as acetate, $C_{30}H_{40}O_7$, m.p. 195-196°; [α]D +175°; λ_{max} 218 nm (∈16, 100); λ_{max} 1739, 1695 and 1220 cm⁻¹.



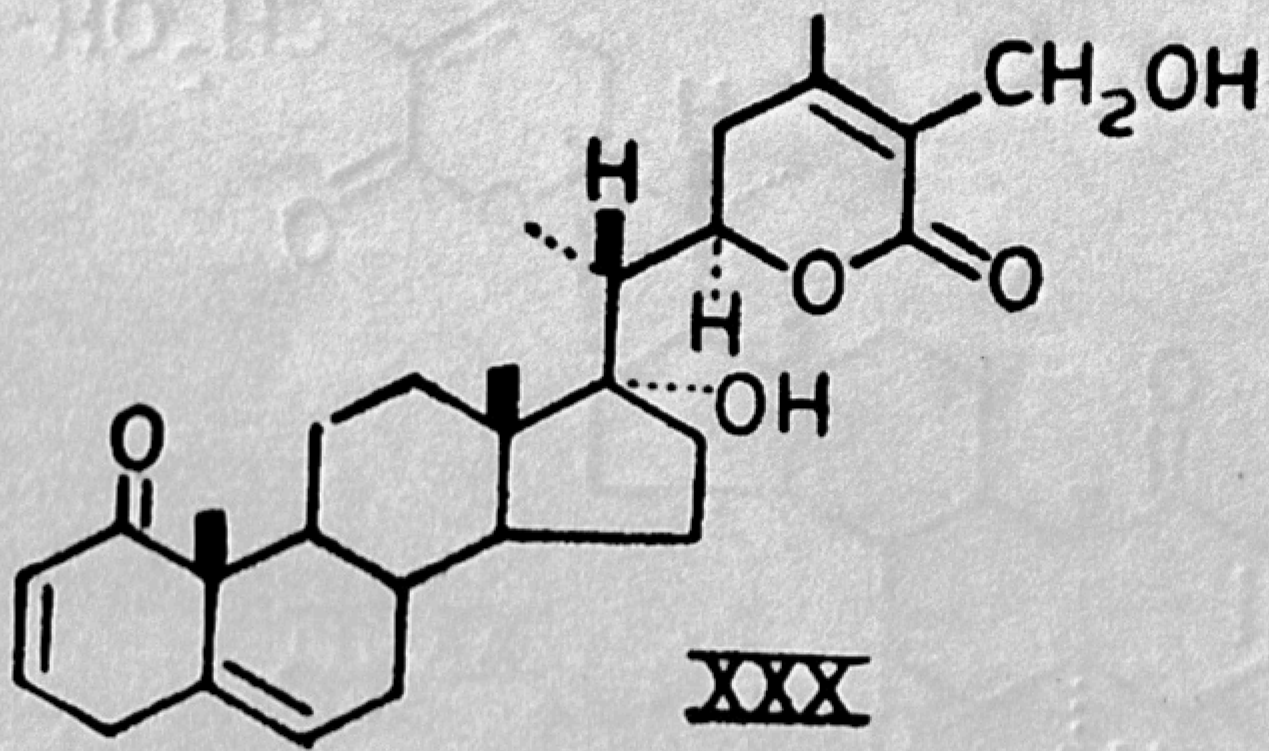
XXIX

The catalytic hy drogenation afforded a dihydroderivative, $C_{30}H_{42}O_7$, m. p. 198°.

The authors have deduced that compounds (XXVIII) and (XXIX) exist in nature as free alcohols, since the mixture of eluates before acetylation showed no signals for an acetoxy-group in the n. m. r. spectrum.

17α,27-dihydroxy-1-oxo-22R-witha-2,5,24-trienolide : (XXX) :-

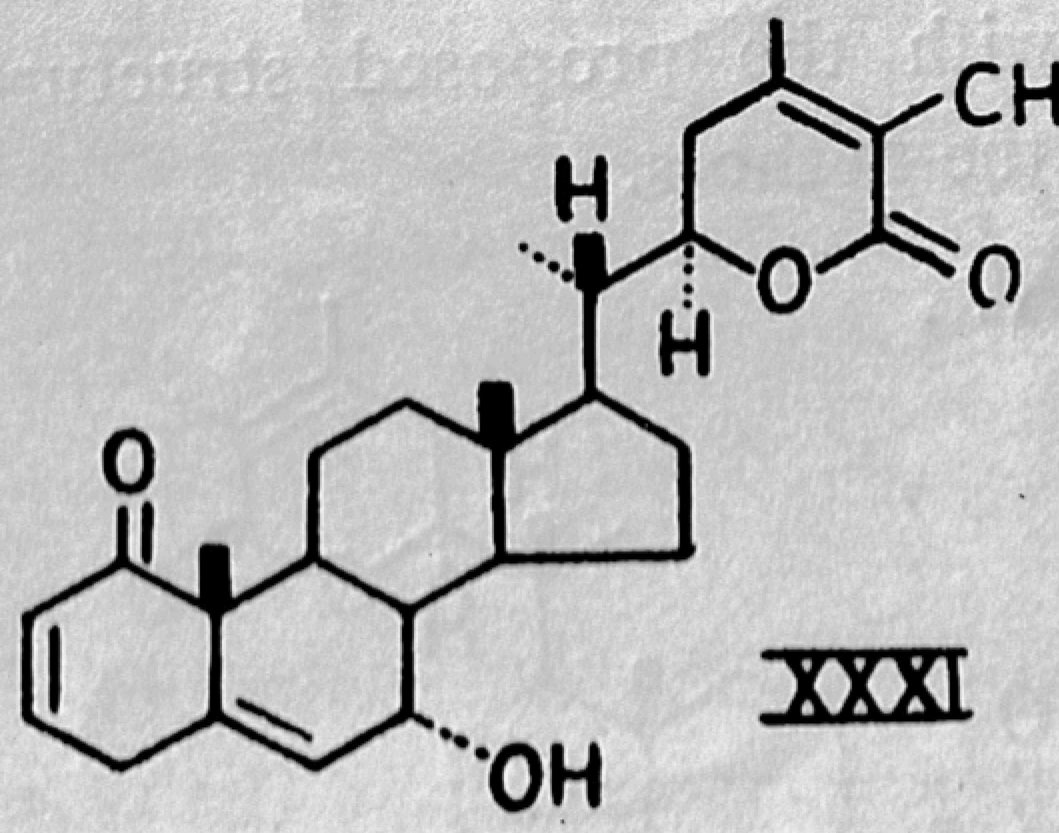
This compound isolated as its monoacetate, $C_{30}H_{40}O_6$, m. p. 205°,,[α]D+74°; λ_{max} 217 nm (∈ 18,000); three bands in the i. r., carbonyl region at 1739 (acetate), 1704 and 1695 cm⁻¹. Hydrogenation of the compound gave a dihydrodeoxyderivative $C_{28}H_{40}O_4$, m. p. 217°.



XXX

7α-2-5dihydroxy-1-oxo-22R-witha-2,5,24-trienolide : (XXXI) :—

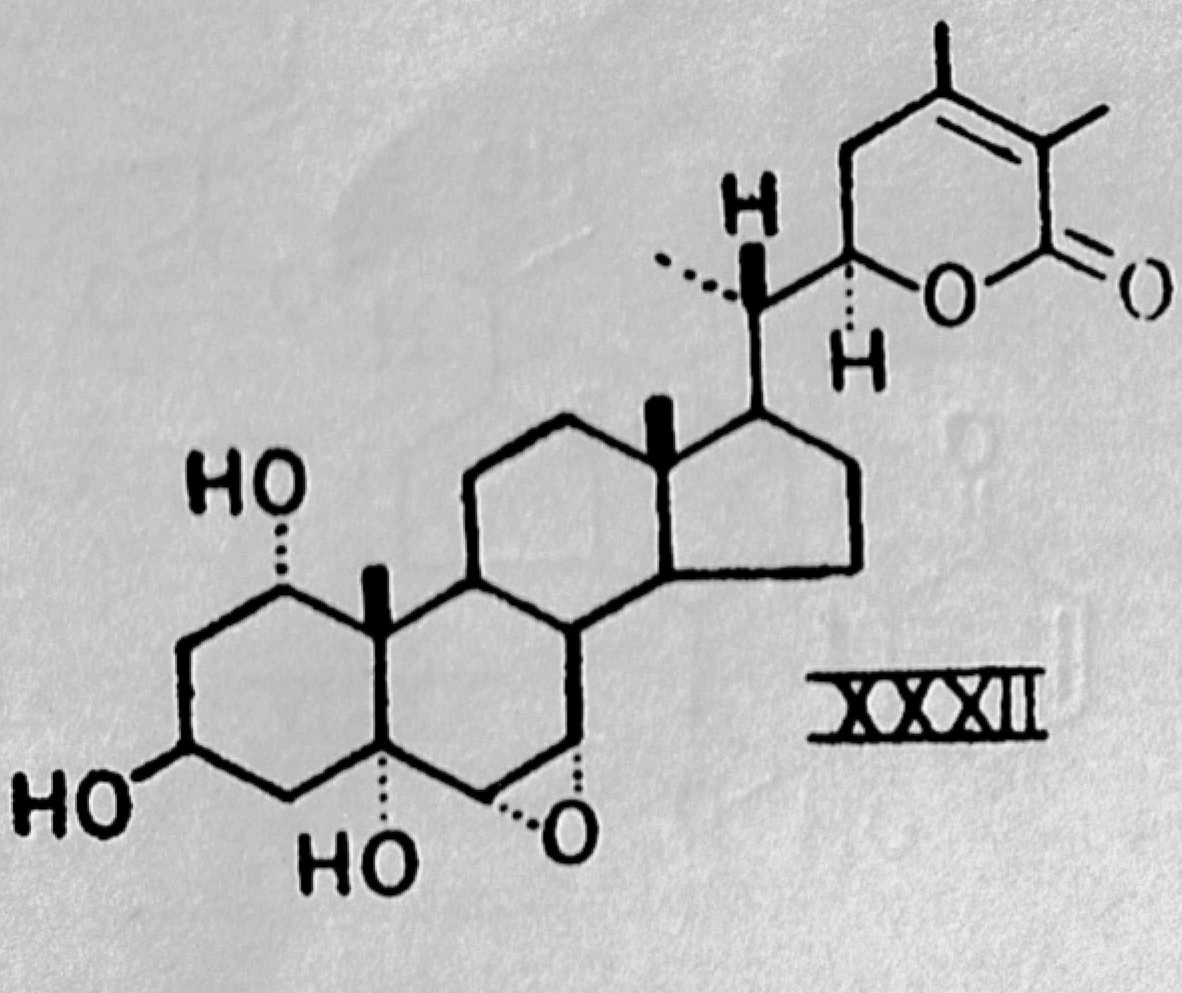
It was isolated as the diacetate, $C_{32}H_{42}O_7$, m. p. 208-209°; [α]D -119°; λ_{max} 216 nm (∈ 18,500); ν_{max} 1739, 1704, 1695 and 1235 cm^{-1}. Epoxidation of this compound with meta-chloroperbenzoic acid yielded 5α, 6α -epoxide, $C_{32}H_{42}O_8$, m. p. 221°. Upon treatment with methanolic potassium carbonate, it gave a trienone, $C_{29}H_{38}O_4$, as yellow crystals, m. p. 213-214°. Under the basic condition of this reaction the allylic 27-acetoxy-group underwent methanolysis to the corresponding methyl ether.



XXXI

1α, 3β, 5α-trihydroxy-6α, 7α-epoxy-22R-witha-24-enolide : (XXXII) :—

This compound was isolated as the corresponding monoacetate $C_{30}H_{44}O_7$, m.p. 256-258°; [α]D+71.5°, λ_{max} 226 nm (ν_{max} 9600); ν_{max} 1736, 1962 cm^{-1}. Oxidation of the compound with the Jones reagent at 0° C gave a ketone, $C_{30}H_{42}O_7$, m.p. 247°.

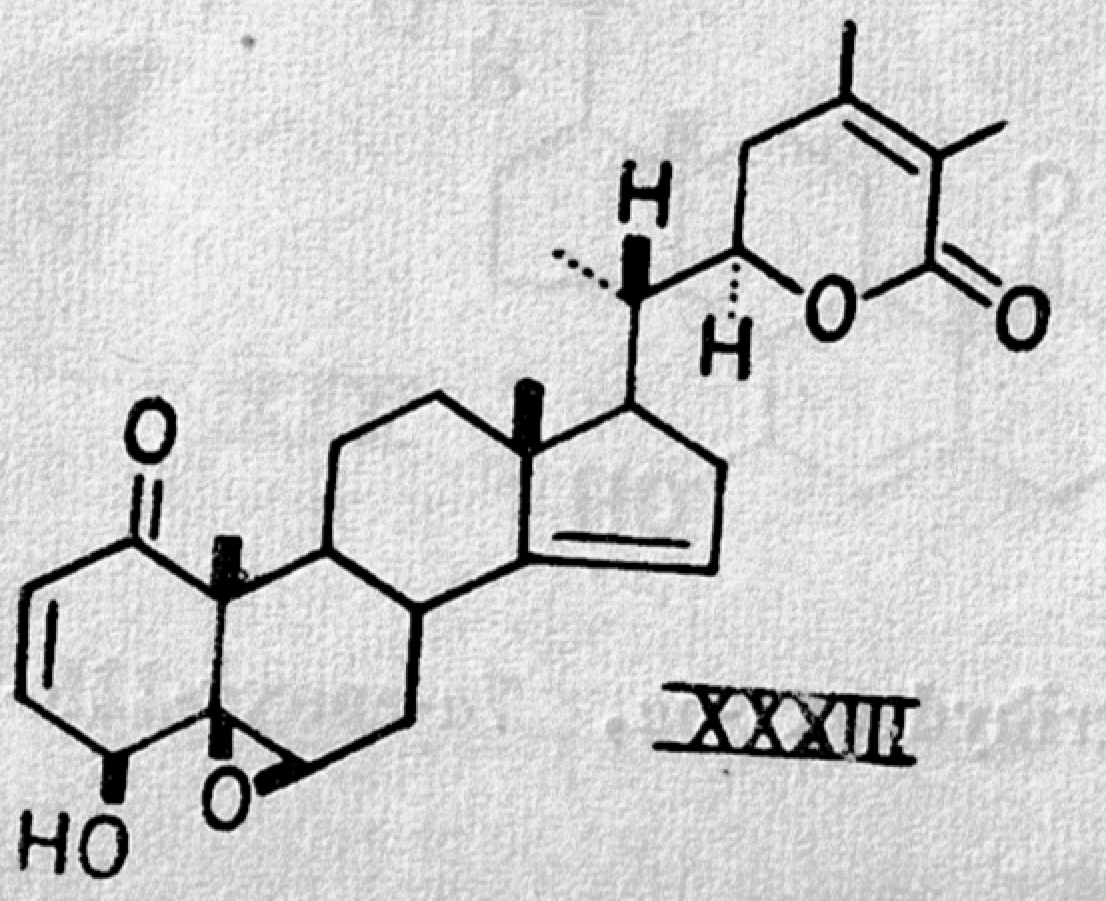


XXXII

4β-hydroxy-5β, 6β-epoxy-1-oxo-22R-witha-2,14,24-trienolide : (XXXIII)—

$C_{28}H_{36}O_5$, m.p. 239-240°; [α]D+122°; λ_{max} 217nm (∈15,500); ν_{max} 1695 and 1661 cm-.¹ Upon acetylation with acetic anhydride and pyridine, it gave a monoacetate, $C_{30}H_{38}O_6$, m.p. 199°, which was found to be identical with the product obtained after the dehydration of the acetate derivative of the compound (XII) with thionyl chloride.

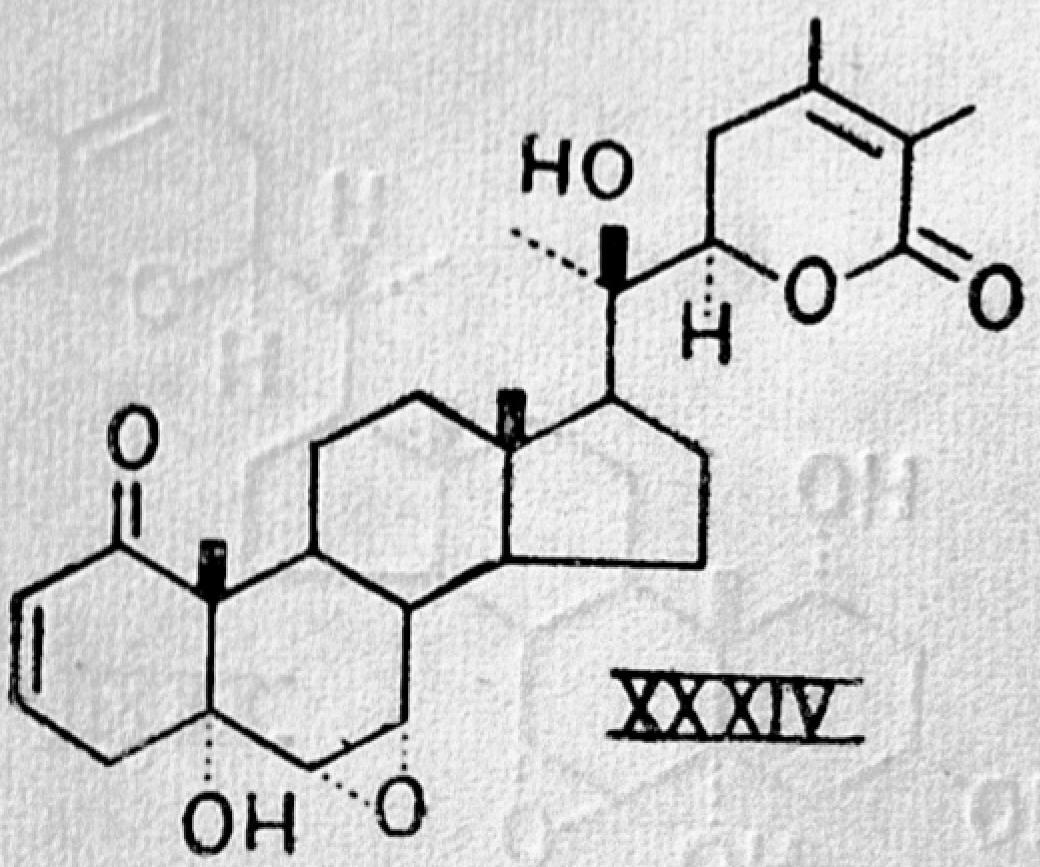
The n. m. r. and mass spectra of all the eight natural withanolides (XXVI to XXXIII) as well as their derivatives were in agreement with the proposed structures and have been discussed in detail by the authors.



XXXIII

5, 20α (R)-dihydroxy-6α-7α-1-oxo(5α) witha-2,24-dienolide : (XXXIV :—

It is another new withanolide which has been isolated Sbyubramanian *et al*[74] from the methanolic extract of



XXXIV

the leaves of *Withania ashwagandha* Kaul. The same authors previously reported the occurrence of this new steroidal lactone from the roots of *Withania coagulans*[75]. The occurrence of this compound from the roots of *Withania somnifera* has also been reported by Mensen *et al*[49].

It is a colourless crystalline compound, $C_{28}H_{38}O_6$, m. p. 281-283°, $(\alpha)D+89°$. It shows u. v. absorption at 223 nm ($\in$14,000) and two bands in the carbonyl region of the i. r. at 5.90 and 5.95μ. Catalytic hydrogenation of the compound gave a 2,3 dihydroproduct, m. p. 290-291°. Upon acetylation with acetic anhydride and pyridine the compound remained unchanged. The structure of this new withanolide was elucidated by its spectral analysis data, derivatives, as well as by direct comparison with the authentic sample isolated from *Withania coagulans*.

The above mentioned 26 new natural steroidal lactones (IX-XXXIV) isolated by different workers, from the leaves of *Withania somnifera*, differ only in their substitution pattern. The compounds (IX-XIII) obtained from chemotype I and II possesses a secondary hydroxyl group at position 4, and a β-oriented epoxide at 5, 6.

The compounds (XIV-XXII) isolated from chemotype III has no substitution at position 4. The only compound in this group which possesses the 5,6-epoxy ring is (XIV), while all others have double bond at this position. Out of the nine withanolides, five (XVI-XX) possess the $\triangle^8$ (41) double bond. This is the first reported occurrence of such compounds in nature. The compound (XXII) is an exception among all the withanolides reported so far, from *Withania somnifera*, in having a α-oriented epoxy ring at 14,15. The two new withanolides (XXIV) and (XXV) isolated from *Withania somnifera*, "South African Type", possess a saturated 6 membered ring lactone in the side chain. This is also the first reported occurrence of such compounds in nature. All the steroidal lactones isolated so far, have a unsaturated (Δ^{24}) 6 membered ring lactone in the side chain. Out of the nine withanolides isolated from chemotype "India I", four (XXVI, XXVIII, XXXII, XXXIV) possesses a α-oriented epoxide at 6,7. Rest of the compounds have substitution pattern similar to other withanolides. Compound (XXIII) is close to (XXI) isolated from chemotype III in having Δ^{14} double bond.

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